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STUDIES

FROM THE

LABORATORY OF PHYSIOLOGICAL CHEMISTRY

SHEFFIELD SCIENTIFIC SCHOOL

OF

YALE UNIVERSITY

For the Years 1887-88

VOLUME III

EDITED BY

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[From Transactions Connecticut Academy, Vol. VIII, Nov., 1888.]

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Some Experiments on the Physiological Action of Uranium Salts.

By R. H. Chittenden and Alexander Lambert, M.D.

In 1885, experiments were commenced in the laboratory of physiological chemistry at Yale University, to ascertain something regarding the physiological and toxical action of uranium salts. At that time there was little accurate knowledge concerning uranium. Gmelin* had, in 1824, performed a few experiments with the nitrate from which he concluded that this salt is a feeble poison; thus he states that 15 grains had no effect on a dog, that 1 drachm merely caused vomiting after more than an hour's interval, and that 34 grains killed a rabbit in 52 hours by stopping the irritability of the heart, while 3 grains injected into the jugular vein of a rabbit caused instant death. Later, in 1851, there appeared a statement in the British and Foreign Medico-chirurgical Review that Leconte always found sugar in the urine of dogs slowly poisoned by small doses of uranium nitrate. This statement was commented upon by Hughes in his manual of pharmico dynamics (p. 866), and has been made the basis of a claim by the so-called homœopathic school that uranium nitrate is a remedy for diabetes. Hughes also refers† to a monograph by Edward Blake on uranium, where three persons and nineteen animals were experimented on. In none of Blake's subjects, however, human or brute, was sugar eliminated in the urine. Ulceration of the pyloric end of the stomach and of the duodenum was found well marked in several of the animals, although in no case was the drug introduced directly into the stomach. Hughes, likewise, refers to several cases of diabetes which he considers were cured by the exhibition of small doses of uranium nitrate,‡ one-sixth to one-third of a grain three times a day. This constitutes all the

Compliments of R. H. Chittenden.

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ism. The results, already published,* show plainly that all of the uranyl salts, with one or two exceptions, have a more or less marked inhibitory influence on amylolytic and proteolytic action. With the salivary ferment, even 0·010 per cent. of uranyl nitrate was sufficient to completely stop all action, while with pepsin-hydrochloric acid and with alkaline trypsin solutions, 0·5 per cent. of the same salt was required to produce an equal effect. In this case the inhibitory action of the uranium salt was, in part at least, due to the formation of a more or less definite and indigestible compound of uranium with the proteid matter to be digested.†

On the excretion of carbonic acid, uranyl nitrate, by a later series of experiments,‡ was also found to have a marked influence. With rabbits, the hypodermic injection of this salt was followed by a slight rise in body temperature and a decided increase in the elimination of carbonic acid. The action of the salt was somewhat slow, but repetition of the experiment always led to an increase of body temperature and a decided increase in the amount of carbonic acid excreted. 0·7 gram of the salt in divided doses was required to produce the result stated, the rabbit not suffering any apparent ill effects from this quantity.

The object of the present series of experiments has been: 1st, to ascertain the influence of uranium salts on proteid metabolism; 2d, to ascertain something regarding the toxic action of uranium salts; and 3d, whether uranium has any influence on the production of glycosuria.

Influence on proteid metabolism.

In this experiment a mongrel bitch weighing 18·8 kilos was employed. The animal was confined in a convenient cage suitably arranged for the collection of the excreta, and was fed during the experiment upon a constant diet of known composition. A large quantity of fresh, lean beef finely chopped, was dessicated at a low temperature until it had lost about 75 per cent. of water. It then contained 11·88 per cent. of nitrogen, as determined by Kjeldahl's method. A large quantity of ordinary soda crackers were obtained and when sampled were found to contain 0·69 per cent. of nitrogen. 40 grams of this prepared beef and 25 grams of the crackers, with

* Chittenden and Hutchinson. Studies from the Laboratory of Physiological Chemistry, Yale University, vol. ii.

† See Chittenden and Whitehouse. Studies, vol. ii, p. 111.

‡ Chittenden and Cummins. Studies, vol. ii.

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400 c. c. of water were fed to the animal twice daily, making a total daily income of 10·054 grams of nitrogen. As soon as nitrogenous equilibrium was established, the 24 hours' urine was analyzed for nine consecutive days, thus giving the average composition of the normal excretion. Uranyl nitrate was then administered for ten consecutive days in gradually increasing quantities.

The accompanying tables give the analytical results. Nitrogen was determined by the Kjeldahl method, sulphur and phosphorus by fusion of a given volume of the urine with potassium hydroxide and potassium nitrate in a silver crucible, and precipitation of the sulphur as barium sulphate and of the phosphorus first as phospho-ammonium molybdate and then as ammonio-magnesium phosphate.*

NORMAL URINE—Without uranium.

Date	Volume.	Re-action.	Sp. Gr.	Nitrogen	Sulphur.	Phos-phorus.	Dose of Uranyl nitrate.
May	c. c.			grams.	gram.	gram.	
31	565	acid	1016	8·414	0·441	0·572	0
June							
1	610	"	1017	10·380	0·516	0·637	0
2	630	"	1016	10·040	0·558	0·665	0
3	600	"	1018	9·618	0·589	0·595	0
4	630	"	1018	10·770	0·661	0·715	0
5	570	"	1019	10·325	0·608	0·632	0
6	460	"	1020	8·738	0·628	0·746	0
7	590	"	1018	10·091	0·576	0·712	0
8	640	"	1016	10·763	0·604	0·645	{ 0·025 gram. 0·025
Total	*5295*	....	....	*89·139*	*5·181*	*5·919*	
Daily average	*588*	....	*1017·5*	*9·904*	*0·575*	*0·657*	

Examination of the first table shows that the animal was in nitrogenous equilibrium, the average daily excretion of nitrogen for the normal period being 9·904 grams while the daily amount of nitrogen taken was 10·054 grams, thus showing a fairly close agreement, espe

* See studies from Laboratory of Physiological Chemistry, Yale University, vol. ii, p. 88.

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cially as there would be a slight loss of nitrogen through the hair shed daily. On commencing the administration of uranium nitrate, small doses were at first given two or three times daily in gelatin capsules, at times not to interfere with digestion. On the second day, uranium was detected in the urine. On June 12th, the 5th day the uranium salt was given, a trace of albumin appeared in the urine and on the day following, the 24 hours' urine contained 1·74 grams of albumin.

WITH URANIUM.

Date.	Volume.	Reaction.	Sp. Gr.	Total nitrogen.	Nitrogen—nitrogen of albumin.	Sulphur.	Phosphorus.	Dose of Uranyl nitrate.
June	c. c.			grams.	grams.	gram.	gram.	gram.
9	600	acid	1017	10·285	10·285	0·587	1·226	0·035 0·035
10	600	"	1015	8·895	8·895	0·404	0·556	0·050 0·025
11	602	"	1018	8·925	8·925	0·574	0·612	0·050 0·025
12	680	"	1017	8·380	8·380	0·525	0·603	0·050
13	640	"	1022	11·391	11·093	0·724	0·621	0·025 0·025 0·025
14	710	"	1020	8·847	8·439	0·634	0·628	0·025 0·050
15	720	"	1023	10·608	10·141	0·571	0·628	0·050 0·050 0·050
16	830	"	1022	11·767	11·446	0·738	0·704	0·075 0·075 0·075
17	640	"	1025	11·178	10·955	0·606	0·626	0·150 0·150 0·150
Total.	*6022*			*90·276*	*88·559*	*5·363*	*6·204*	*1·295* grams.
Daily average	*669*		*1019·9*	*10·031*	*9·839*	*0·596*	*0·689*	
18	650	acid	1026	10·179	9·772	0·611	0·542	0

On the 16th of June, the urine contained 7·7 grams of sugar. The appearance and disappearance of both albumin and sugar were followed quantitatively and the results will be discussed later on. Naturally, the appearance of albumin in the urine compels a correction in the table of nitrogen results on those days when albumin was excreted. This has been done by subtracting from the total nitrogen found, the nitrogen equivalent to the albumin, on the assumption that the latter contains 15·5 per cent. of nitrogen.

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Examination of the analytical results shows that the uranium salt has a marked influence on the excretion of water, the increase amounting on an average for the nine days period to over 80 c. c. per day. There is also a very marked increase in the specific gravity of the daily excretion, but this is without doubt in great part due to the presence of albumin and sugar, as the increase is most noticeable on those days when the largest amounts of sugar and albumin were excreted.

Regarding nitrogen, a comparison of the total amounts excreted for the two periods, after correction for the nitrogen of the albumin, indicates that small doses of the uranium salt have little, if any, influence on proteid metabolism. On the last three days of the uranium period, however, when the amount administered had not only been increased, but there was doubtless also an accumulative effect, the excretion of nitrogen appeared to be considerably above the normal, certainly enough to warrant the assumption that comparatively large doses of uranium salts may increase somewhat proteid metabolism, and this view is sustained by the increase in both the total and daily average amounts of sulphur and phosphorus excreted. During the uranium period of ten days, 1·295 grams of the nitrate, or 19·98 grains, were given without any apparent ill effects being immediately produced.

Excretion of sugar and albumin.

After about 0·4 of a gram of uranium nitrate had been given, the urine began to show traces of albumin and five days after this, sugar made its appearance. The amounts excreted are shown in the accompanying table. Sugar was determined by Allihn's gravimetric method, and albumin by boiling with acetic acid and collecting the coagulum on a weighed filter. On the 18th, the weighed diet was discontinued, but the urine was kept under close scrutiny with the results shown in the table. The sugar first disappeared and four days after this the albumin, likewise. On again administering uranium, and in much larger doses than in the first series, both sugar and albumin failed to appear until after a single dose of over 4 grams of the salt had been administered, when considerable albumin showed itself in the urine. We were then compelled to stop the experiment, and the dog was chloroformed and a post-mortem immediately made.

The liver was excessively congested and appeared abnormally large. Microscopic examination of hardened sections showed an

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TABLE SHOWING THE AMOUNTS OF ALBUMIN AND SUGAR IN THE DOG'S URINE.

Date.	Volume.	Reaction.	Sp. Gr.	Albumin.	Sugar.	Dose of Uranyl nitrate.
June	c. c.			grams.	grams.	grams.
8	640	acid	1016	0	0	0·050
9	600	"	1017	0	0	0·070
10	600	"	1015	0	0	0·075
11	602	"	1018	0	0	0·075
12	680	"	1017	trace	0	0·050
13	640	"	1022	1·741	0	0·075
14	710	"	1020	2·634	0	0·050
15	720	"	1023	3·009	0	0·150
16	830	"	1022	2·066	7·735	0·225
17	640	"	1025	1·441	7·449	0·450
18	650	"	1026	2·633	7·476	0
		weighed diet discontinued.				
19	1280	acid	1016	3·604	14·387	0
20	....	"	....	considerable	considerable	0
21*	750	"	1027	3·048	0·525	0
22	410	alkaline	1035	1·756	0	0
23†	....	acid	....	some	0	0
24	460	"	1018	0·578	0	0
25	430	alkaline	1030	trace	0	0
26	....	....	....	0	0	0
27	....	....	....	0	0	0·4
28	....	....	....	0	0	0·5
29	....	....	....	0	0	0
30	....	....	....	0	0	0
July 1	....	....	....	0	0	1·0
2	....	....	....	0	0	0
3	....	....	....	0	0	1·5
4	....	....	....	0	0	4·4
5	....	....	....	considerable	0	0

infiltration of cells around the blood vessels, but aside from this and an apparent tendency of the liver cells to separate into stringy masses there was nothing abnormal. The kidneys both had adherent capsules and the medullary portion was large in ratio to the cortex, which, besides being smaller than normal, was striated. The lesion as made out microscopically was acute parenchymatous neph-

* On this day granular casts were found, but they were not present on the following day.

† Dog vomited freely.

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ritis. The endothelial cells of the malphigian tufts were swollen and proliferated, so that frequently the tuft of vessels was compressed by these proliferating cells and by the detritus and infiltrating pus cells. In the convoluted tubules the cells were swollen, granular and very much broken down, while the lumen of the tubes themselves was often filled with detritus and cast matter. In the straight tubules, the cells were also occasionally broken down and frequently swollen and granular. The stroma was found in places infiltrated with pus cells. Stomach and intestines were normal.

Further data regarding the excretion of albumin and sugar, through the action of uranium, are given in the description of the toxic action of this substance.

The toxic action of uranium.

Our experiments on the toxic action of uranium have been confined wholly to the action of pure uranyl nitrate on rabbits, nine in number. The animals were confined in cages with proper outlets for collecting the excreta, and were fed on grass, spinach, and other green food.

Experiment I.

Large, vigorous buck. In this experiment small doses of uranium, gradually increased, were given in gelatin capsules, and the urine examined each day for albumin and sugar. The accompanying table gives the details of dose and the amounts of sugar and albumin found.

Outside of changes in the urine there were no indications of toxic action until on the ninth day, when a slight weakness was noticeable, especially in the hind legs. After this, the animal gradually grew weaker and emaciated, with total loss of appetite and with eyes dull and watery. On the 8th of June, a large quantity of thick tenacious mucus mixed with pin-head fæces was passed. Motion of all kinds was difficult. On the 9th of June, the animal could not sit up and during portions of the day there appeared to be paralysis of both hind legs. Later in the day the power of motion returned, and when placed on his back the rabbit could kick out feebly with both legs. On the next day his weakness and dullness were still more pronounced, and when stirred from a squatting posture he trembled violently. His eyes were watery and glazed, with the pupils widely dilated. On touching the cornea he did not offer to close the eyelids, but endeavored to withdraw his head. All power of vision was apparently lost. Later, he lost the power of coördination in his legs, was unable to make any coördinate movements, though still retaining

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the power to move each limb separately. All fæcal discharges had stopped and the animal refused all food.

Date.	Volume.	Reaction.	Sp. Gr.	Albumin.	Sugar.	Dose of Uranyl nitrate.
May	c. c.			gram.	gram.	gram.
25	73	alkaline.	1020	0	0	0·025
26	—	"	—	0	0	0·050 0·025
27	70	"	1020	0	0	0·050
28	85	"	1016	0	0·035	0·050
29	75	"	1022	0·189	trace.	0·050
30	—	—	—	—	—	0·050
31	75	"	1023	0·140	0·492	0·050 0·025
June 1	82	"	1023	0·125	0	0·050 0·050
2	75	"	1021	0·101	0	0·050 0·050
3	80	"	1020	0·173	0	0·050 0·050
4	18	neutral.	—	0·217	0	0·050 0·050
5	12	alkaline.	—	0·155	0	0·050
6	—	—	—	—	—	0·100
7	45	"	1025	0·518	0	0·100 0·100
8	30	"	—	0·301	0	0·150 0·150
9	20	"	—	considerable.	0	0·200 0·200
10	0					0·200 0·150
						2.175 grams.

On the morning of the 11th found dead. Post-mortem showed the heart distended on the right side and the lungs normal. The liver was much smaller than normal and the gall-bladder full of very black bile. On a microscopic examination of the liver, the only noticeable feature was the extreme congestion. In the stomach, the mucous membrane seemed somewhat disintegrated. In the intestines, brown colored spots were seen where Peyer's patches are situated, and microscopic examination showed that the latter were deeply inflamed. Further, from the large amount of mucus in the mucous glands, it was evident that there was acute catarrhal inflammation of the intestines. The cæcum was full of black matter. There were no normal fæces in the colon or rectum, only the pin-head variety.

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The kidneys were very much diminished in size and extremely congested. Sections showed that the epithelial cells of the malpighian tufts were not swollen or proliferated, though in some places the tufts of vessels were slightly compressed by detritus. The cells of the convoluted tubules were swollen, granular and broken down or gone entirely, as were also the cells of the straight tubes, the latter being also filled in places with casts and detritus. The vessels between the tubes were filled with blood. The stroma was swollen and infiltrated with pus cells and in places there were extravasations of blood.

Brain and cord were slightly congested, but otherwise normal.

Examination of the table of urine tests shows that sugar appeared first in the urine, but continued only for three days, while albumin showed itself immediately after the first appearance of the sugar and continued throughout the experiment. The amount of urine voided grew gradually smaller as the animal began to feel the full effects of the uranium.

Experiment II.

In this experiment, uranium was given as indicated in the following table, and the urine examined each day.

Date.	Volume.	Reaction.	Sp Gr.	Albumin.	Sugar.	Dose of uranyl nitrate.
June	c. c.			gram.	gram.	gram.
1	—	alkaline.	—	0	0	0·050
2	100	"	1014	trace.	0	0·050 0·050
3	55	"	1025	0·192	0	0·050 0·050
4	41	"	—	0·227	0·406	0·050 0·050
5 6	125	acid.	1024	1·095	1·078	0·050 0·050
7	120	alkaline.	1024	0·523	0·917	0·050 0·050
8	100	acid.	1022	0·237	trace.	0·100 0·100
9	55	alkaline.	—	0·351	0	0·100 0·100
10	125	"	1022	0·687	0·430	0·100 0·100
11	75	"	—	considerable.	0	0·100
12	75	"	1022	0·332	0	0·100
						1.350 grams.

On June 4th, the animal began to show weakness, particularly noticeable in his hind legs, but this disappeared and the animal

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appeared better. On the 11th, it had lost its appetite, become emaciated and showed general weakness and loss of muscular power. On the following day at noon it was found dead, without having shown any marked symptoms other than general depression of the muscular system.

The heart was found greatly distended on the right side, lungs normal and the liver small and congested, especially near the lower edges of the left lobe. Kidneys were also small and congested. The bladder contained quite a little urine, with considerable albumin and sugar in it.

Experiment III.

This experiment was practically a duplicate of the preceding. An albino buck was used and the uranium salt was forced down the throat in gelatin capsules, as in the preceding experiments. The table shows the amount of nitrate given and the character of the urine each day.

Date.	Volume.	Reaction.	Sp. Gr.	Albumin.	Sugar.	Dose of uranyl nitrate.
May	c. c.			gram.	gram.	gram.
25	171	alkaline.	1014	0	0	0·025
26	—	acid.	—	trace.	0	0·050 0·025
27 28	no urine passed.					0·050
29 30	75	alkaline.	1026	0·494	0.275	0·050 0·050
31	no urine passed.					0·050 0·025
						0·300 gram.

The first symptom noticeable was the suppression of urine for over forty-eight hours. On the 27th, the animal was quite dull and showed signs of general weakness. As he walked, he moved as if it caused him pain in the lumbar region. On the next day he showed great thirst, and he finally died on June 1st, a loose diarrhœa setting in a few hours before death.

Post-mortem examination showed that the heart had stopped in diastole, and that it was much distended with blood. Lungs were normal. The liver was congested, as were also the kidneys. The latter had non-adherent capsules. The stomach contained considerable undigested food, and its mucous membrane was partly disintegrated. Portions of the duodenum were somewhat congested. The

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bladder contained a few cubic centimeters of urine, which had in it considerable sugar and some albumin.

The most noticeable feature of this experiment was the suppression of urine.

Experiment IV.

In this experiment, a large white buck was used and the uranium was administered in capsules as follows:

May 17	0·050	gram	uranyl	nitrate.
18	0·075	"	"	"
19	0·075	"	"	"
20	0·025	"	"	"
	0·225			

There were no symptoms of toxic action until the morning of the 20th. The rabbit was then dull and weak. He trembled violently as he hopped about; the hind legs appeared partly paralyzed. The pupils were dilated and the appetite gone. On the 21st, weakness was still more pronounced. Any movement was accompanied by tremblings and great difficulty was experienced while walking, in making the different movements correctly. There was severe diarrhœa and the animal showed extreme emaciation. On the 22d, the diarrhœa had passed into an involuntary defecation more or less continuous. The animal was too weak to move and lay all the forenoon breathing heavily, though the number of respirations per minute did not go much beyond normal, 45–54 per minute. On the afternoon of this day, two days after the last dose of uranium had been administered, the animal appeared stronger, the involuntary defecations had ceased, though a loose diarrhœa still continued, and the animal appeared to have recovered the use of its locomotive muscles. On the 23d, however, the animal was again unable to move and finally died at noon, the diarrhœa having continued more or less up to death.

During the last three days there was complete suppression of urine, but on the 20th, 59 c. c. of urine were passed, of specific gravity 1022, and which contained 0·656 gram of sugar.

On post-mortem examination, the heart was found to have stopped in diastole and engorged with blood. The lungs were congested. The kidneys had non-adherent capsules and the cortex and medulla showed severe congestion. The stomach contained no food, but considerable tenacious mucus. The small intestines from duodenum to cæcum were very much congested.

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Experiment V.

Gray and white buck. This experiment was a repetition of No. IV, the same amounts of uranium salt being given and at the same intervals of time. The animal died three days after the last dose of uranium had been administered. The symptoms were not very marked, simply loss of muscular power, and a gradual wasting away. On post mortem, the heart was found in diastole and engorged with blood. Lungs congested. The liver was black with the blood in it, especially on the edges, and when pressed between the fingers was extremely pliable and of a soft, pulpy consistency, showing marked parenchymatous degeneration. The gall bladder was distended with very dark bile. Both kidneys had non-adherent capsules. The left kidney was slightly congested. The intestines had an inflamed patch in the jejunum and were congested for a foot above it. The bladder contained a little urine, which gave reactions for both sugar and albumin. Sugar and albumin were also found in the urine several days before death.

Experiment VI.

In this experiment still smaller amounts of uranium were administered, a total of only 0·175 gram being given in three days. The following table shows the dosage and the changes in the urine:

Date.	Volume.	Reaction.	Sp. Gr.	Albumin.	Sugar.	Dose of uranyl nitrate.
June	c. c.			gram.	gram.	gram.
14	—			0	0	0·050
15	—	alkaline	1043	0	0	0·050 0·025
16	110	"	1027	0·104	0	0·050
17	75	"	1023	0·337	0	
18	30	"	—	0·126	0	0·175
19 20	60	"	—	0·154	0	
21	—	"	—	some	0	
22	50	"	1015	trace	0	

There were no noticeable symptoms of toxic action aside from the changes in the urine, and even here there was no sugar at any time present. Just as the albumin had almost disappeared from the urine and we looked for speedy recovery, the animal was found dead (on the 23d). Post-mortem showed the heart in diastole, engorged with blood. Lungs normal. Liver normal, aside from a slight congestion.

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Sections under the microscope showed no pathological changes. The kidneys were congested. Under the microscope, the epithelial cells were proliferated and occasionally the tufts were seen slightly compressed by these proliferating cells and detritus. The convoluted tubules showed swollen and granular cells, even broken down, in places. In the straight tubules, the cells were also swollen and granular and sometimes were detached from the tubes. In places, there was cast matter and detritus in the tubes. The stroma was normal. Hence, here, as in many of the preceding experiments, it is a case of acute parenchymatous nephritis. The stomach was full of undigested food and apparently normal; the duodenum was congested and the small intestines throughout were nearly empty, except for a little mucus. The bladder had in it about 10 c. c. of urine, which contained a trace of albumin but no sugar.

Experiment VII.

An albino rabbit. A single dose of 0·3 gram of uranyl nitrate was given in a gelatin capsule by mouth on June 13th. Outside of changes in the urine there were no symptoms whatever until the 17th, when in the early morning the animal was found weak and powerless, all motor power completely gone. It rapidly grew weaker and died in the afternoon of the same day.

The right side of the heart was found much distended. The lungs were normal, the liver small and congested. Kidneys were also small and congested and of a cloudy appearance. Microscopic examination showed acute parenchymatous nephritis. Stomach was full of undigested food, but the intestines were empty. Both appeared normal.

On June 15th, 160 c. c. of alkaline urine were passed, of specific gravity 1023. It contained 0·748 gram of albumin and 1·069 grams of sugar. On the 16th, 30 c. c. were passed of 1022 specific gravity and containing 0·412 gram of albumin and 0·354 gram of sugar. On the 17th, no urine was passed and on making the post mortem the bladder was found empty.

With this rabbit, an attempt was made to ascertain how much carbohydrate matter there was in the liver at the time of death. 40 grams of the sampled and finely ground liver were thoroughly extracted with boiling water (continuous extraction for three days), frequently replaced. The several decoctions were ultimately united and finally brought to a volume of 500 c. c. Two portions of 200 c. c. each were placed in suitable flasks and sufficient hydrochloric acid

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added to each, to make the fluid contain 2 per cent. HCl. The acid fluids were then heated on water-baths for 15 hours, in order to convert all carbohydrate matter into dextrose. The fluids were then neutralized, evaporated and finally tested for dextrose with Fehling's solution, by Allihn's gravimetric method. Both solutions failed to give any reducing action whatever, thus showing a total lack of carbohydrate matter in the liver. This is in strong contrast to the normal condition of a rabbit's liver, which contains an abundance of carbohydrate matter, both sugar and glycogen; on an average 10·35 per cent. of total carbohydrates, as determined by methods similar to the one just described.* Glycogenic function is then destroyed by uranium, the same as in phosphorus poisoning, but unlike the action of phosphorus there is apparently no fatty degeneration of the organs.

The liver here experimented with was small for the size of the rabbit, a large white buck. It was also noticeable, as in many of the other rabbits experimented on, that while the stomach was full of food, sometimes even distended by it, there was nothing at all in the intestines below the pylorus until the cæcum was reached, which was again full, and the intestines below were either almost free from fæces or else contained only the pin-head variety. The food in the stomach was wholly undigested. As has been already pointed out, a small percentage of a soluble uranium salt is sufficient to completely stop gastric and pancreatic digestion. This being the case it is quite probable that the emaciation, etc., so noticeable in uranium poisoning, is the direct result of the action of the salt on the digestive functions. Nothing being digested there would be no matter for absorption, and hence no sugar-forming material for the liver. All the carbohydrate matter stored up would in a little time be completely consumed and as the portal blood could bring no new nutritive matter, the liver would naturally diminish in size and the animal become emaciated and eventually die from that cause alone, even if the uranium gave no other direct cause of death. In this connection we also need to recall the general increased metabolism of both nitrogenous and non-nitrogenous matter, under the influence of uranium.

The uranium salt may also act specifically on the liver cells, affecting their metabolic power, preventing any storage of carbohydrate matter or more probably causing a degeneration of the cells, by which they may be led to give up to the blood in abnormal abundance all the carbohydrate matter previously stored up. This possible

* Post-mortem formation of sugar in the liver. Studies from Laboratory of Physiological Chemistry, Yale University, vol. I, p. 171.

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specific action of the poison on the liver cells would in a measure explain the temporary glycosuria, which appears and then disappears so frequently in uranium poisoning. Further, by a combination of this specific action with the non-absorption of nutritive matter through retarded digestion, it would be easy to explain the alternate appearance, disappearance and re-appearance, of sugar in the urine in these cases. Where the symptoms run their course with a fair degree of rapidity, as in continued dosing with uranium, sugar appears in the urine for a few days and then disappears, although the amount of uranium administered may be steadily increased. In fact, the increased dose of uranium is doubtless the cause of the further non-appearance of the sugar, since the stored up carbohydrate material having been wholly used up, and at the same time digestion and absorption being prevented, there is no more carbohydrate-producing material available, either directly or indirectly. On the other hand, if, after the sugar at first present has disappeared from the urine, there comes an interval of a day or so when uranium is not administered and digestion again starts up, then the specific action may again come into play and a temporary glycosuria again result. This condition was noticed in experiment No. II.

In some of the experiments, there seems to have been a visible change in the hepatic cells, as in the experiment next to be described, where under the microscope the cell bodies appeared as if collected into small granules or even broken down. Again, in some experiments, as in No. V, there was a noticeable pulpy degeneration of the liver cells.

Experiment VIII.

In this experiment, the uranium salt was introduced by hypodermic injection. 0·23 gram of uranyl nitrate in a little water was injected beneath the skin of the leg of a good-sized rabbit, on June 20th at 4.0 P. M. Two days after he showed marked weakness, although his appetite remained good. On the following day he appeared quite dormant and could be roused only with difficulty; power of motion seemed to be nearly gone. He died the next day at noon. On June 21st, the day following the administration of the uranium, 60 c.c. of alkaline urine were passed containing 0·234 gram of albumin and 0·660 gram of sugar. After this, there was complete suppression of urine till death.

Post-mortem.—Heart had stopped in diastole; lungs normal. The liver was mottled and congested slightly in areas. Under the micro-

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scope, sections showed the cell bodies strongly granulated, with their outlines mostly clear, but in places broken down.

There was also an infiltration of pus cells around the blood vessels. The kidneys were normal in size and appearance, with non-adherent capsules. Under the microscope, however, all the conditions characteristic of acute parenchymatous nephritis were to be seen. The epithelial cells of the malpighian tufts were proliferated, so much so as to compress the vessels. Both convoluted and straight tubules had their cells swollen, granular and broken down. The stroma was normal. Some adipose tissue in the pelvis of the kidney was infiltrated with pus cells. There was also considerable detritus between the papillæ. The bladder was empty and contracted. In the abdominal cavity there was about 30 c.c. of a coagulable, clear fluid. The stomach was full of food, the intestines empty. No fæces in colon and rectum. No signs of congestion in the alimentary tract, except in the rectum, where there were red blotches which proved to be the beginning of inflammation and infiltration of pus cells. The animal appeared to have died from suppression of urine.

Experiment IX.

In this experiment, one large dose of uranium (1·0 gram of the nitrate) was given by mouth on June 27th, at 4.30 P. M. On July 5th. the animal was still alive, but weak and emaciated. There were no symptoms other than those already described. Owing to lack of time we were not able to continue the experiment, but wishing to see any changes which might have occurred in the organs the animal was chloroformed and a post mortem made. On June 28th, the urine passed contained some albumin, but no sugar; on July 1st, considerable albumin, but still no sugar.

The liver was found to be small but not congested. The cell substance, as seen under the microscope, was collected into small granules. Nuclei and nucleoli were quite distinct. Kidneys had non-adherent capsules, but they were congested and the cortex looked striated. Under the microscope, the epithelial cells of the glomeruli were seen to be swollen and proliferated. In the convoluted tubes, the cells were slightly swollen and granular and occasionally broken down. The straight tubes were also broken down in places and contained cast matter. The stroma was normal. Stomach was filled with food, while the intestines were entirely empty. Peyer's patches were swollen and infiltrated with pus cells. The cæcum was partially filled with matter and the end of it looked honey-combed. It

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proved to be slightly inflamed, and with cells infiltrated into it more or less. Colon and rectum were empty.

From these results, collectively, it is to be seen that uranium is an irritant poison and, like other metallic irritants, produces gastro-intestinal irritation of more or less intensity, as shown by the acute diarrhœa and other symptoms met with in this form of poisoning. In the majority of cases, the action of the uranium salt on the intestines results in a simple enteritis, but this is liable to pass eventually into acute catarrhal inflammation.

As ordinarily administered, it is not in any sense a rapid poison; the ingestion of a fatal dose of a uranium salt is not followed by any noticeable effects for some time. The action of a small amount (150 milligrams) is apparently as rapid and pronounced as that of large quantities (1 gram). The first noticeable symptom in rabbits is general weakness, lack of motor power, loss of coördination and occasional temporary paralysis of the locomotor muscles. Introduced into the stomach in sufficient amounts, it checks digestion and even stops it altogether. On the other hand, it appears to increase somewhat proteid metabolism and also to increase the elimination of carbonic acid and raise the body temperature. Hence, it is to be considered as having a direct action on nutrition, the disturbance of which is also plainly indicated by the rapid emaciation which follows the administration of uranium.

Its most marked lesions are its destructive action on the kidneys, and its destruction of the kidney tissue itself. It causes here an acute parenchymatous nephritis of the same kind as found in arsenic, mercury, and phosphorus poisoning. Further, the quantity of albumin found in the urine shows plainly how greatly the blood vessels are involved in the inflammation. The albuminuria produced is severe and constant, and when the uranium is given in a single large dose, as in experiment No. IX, or in a small dose by hypodermic injection, as in experiment No. VIII, then albumin may appear in the urine within 24 hours. The uranium must have some specifically destructive action on the kidney epithelium cells, causing them to swell and break down. At first, with small doses of uranium, the urine is decidedly increased in volume, but later on, when toxic action is more pronounced, there may be a partial or even complete suppression of the urine. This latter condition is naturally more quickly produced by large doses of uranium. In several cases, suppression of urine would seem to have been the cause of death. The urine, too, in a short time after the admin-

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istration of uranium contains more or less sugar; as a rule the sugar does not make its appearance until after the albumin. Salkowski has shown that mercury will also cause diabetes in rabbits, but it is questionable whether it is as constant a symptom as in uranium poisoning. It is also stated that in phosphorus poisoning the urine sometimes contains sugar.* In the hypodermic injection of uranium, experiment No. VIII, sugar appeared in the rabbit's urine within 24 hours. The production of glycosuria is a very characteristic symptom in uranium poisoning. The urine also contains invariably a large amount of crystallized calcium oxalate, which would also point to decided malnutrition and help explain the marked emaciation so commonly seen.

In those cases where the poisoning becomes in a measure chronic, the nervous symptoms sometimes predominate, as shown in loss of sight and power of coördination.

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* H. C. Wood. Therapeutics, p. 110.

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Elastin and the Elastose Bodies. By R. H. Chittenden and Horace S. Hart, B.A., Ph.B.

Elastin, the basis of the so-called elastic tissue, has generally been considered one of the most indigestible of the albuminoid substances. In fact, the older writers looked on it as almost insoluble in the digestive juices. Recently, however, the experiments of Etzinger,* Horbaczewski,† and Morochowetz,‡ have shown that both the *ligamentum nuchæ* of the ox when finely divided, and purified elastin when powdered, are fairly soluble both in pepsin-hydrochloric acid and in natural gastric juice, as Horbaczewski's observations on a man with stomach fistula have fully indicated.

It has been our object, therefore, to study somewhat in detail the primary cleavage products of elastin, so far as they may be formed, making use of the methods§ which have yielded such fruitful results with many of the proteid bodies.

Preparation of Elastin.

As usually described, elastin is a body free from sulphur, insoluble in water even after several days boiling, likewise in cold dilute alkali, acetic acid, dilute hydrochloric acid, alcohol, and ether. Naturally, therefore, the method of preparing such an insoluble body has consisted in removing from a tissue rich in elastin, all extraneous substances by successive treatment with the above-mentioned reagents. In fact, so vigorous is the method of treatment, as usually described, that it appears almost questionable whether a body belonging to a group noted for ease of decomposition might not suffer some change in such a long process of preparation.

We have employed two methods, the first one of which is practically identical with that followed by Horbaczewski. The neck bands of a number of recently killed oxen were thoroughly freed from all adhering fat and muscle and then chopped quite fine; they weighed

* Zeitschrift für Biologie, Band x, p. 84.

† Ueber das Verhalten des elastins bei der Pepsinverdauung, Zeitschrift für physiologische chemie, Band vi, p. 330.

‡ Verdauungsgesetze. Abstract in Jahresbericht für Thierchemie, 1886, p. 271.

§ Kühne and Chittenden, Zeitschrift für Biologie, Band xx, xxii; also Studies from Laboratory of Physiological Chem. Yale University, vol. ii.

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2400 grams. The mass was boiled for four days in water frequently changed, after which it was soaked for 45 hours in 8 litres of 1 per cent. potassium hydroxide, and then boiled with the solution for 4 hours. The tissue was freed from alkali by thorough washing with water, and was then boiled with water, frequently changed, for 16 hours. The potassium hydroxide solution on neutralization, gave a heavy precipitate slightly soluble in excess of 0·4 per cent. hydrochloric acid, easily soluble in strong acid. The addition of acid in excess caused a strong odor of hydrogen sulphide to be given off.

After boiling the tissue a second time with water, it was placed in 8 litres of 10 per cent. acetic acid and warmed for an hour and a half, after which it was allowed to stand in the cold for 16 hours. It was then boiled for 4 hours in the same acetic acid solution, and afterwards washed thoroughly with water. The tissue was then placed in 10 litres of 5 per cent. hydrochloric acid and allowed to soak for some time in the cold, after which it was washed in running water and macerated in water, frequently changed, for 24 hours to take out all traces of the acid. After this treatment, the tissue was boiled with successive portions of water for about 45 hours, when it was placed in a large volume of 95 per cent. alcohol and allowed to remain for several days. It was afterwards boiled in alcohol for about 15 hours and then placed in an excess of ether, after which it was extracted with warm ether for two days.

ANALYSIS OF ELASTIN A.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash %
						c. c.	T. °C.	Pressure. mm.			
I	0·4250	0·2745	7·17	0·8381	53·77	----	----	----	----	----	----
II	0·3398	0·2213	7·23	0·6697	53·74	----	----	----	----	----	----
III	0·5454	----	----	----	----	72·7	8·6	771·5	16·47	----	----
IV	0·4440	----	----	----	----	59·8	9·0	771·2	16·59	----	----
V	0·5125	----	----	----	----	69·4	9·4	767·1	16·59	----	----
VI	0·2971	----	----	----	----	----	----	----	----	0·0027	0·9

Percentage composition of ash-free substance:

						Average.
C	54·26	54·22	----	----	----	*54·24*
H	7·24	7·30	----	----	----	*7·27*
N	- --	----	16·74	16·76	16·62	*16·70*
O	----	----	----	----	----	*21·79*
						100·00

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The tissue on being freed from water was hard, tough, and difficult to powder. It was finally ground to a coarse powder and then re-extracted with warm ether, until the ether on evaporation left no appreciable residue. This took some time, for, as previously pointed out by Horbaczewski, there apparently remains in the tissue a small amount of fat-like matter, which dissolves very slowly in ether and which can be completely extracted only by first grinding the purified tissue as fine as possible. The elastin prepared in this manner we have termed for convenience *Elastin A.*

A portion of the preparation dried at 110° C. gave on analysis the results contained in the accompanying table. The methods of analysis were the same as those previously described in former articles on the proteoses.* By fusion with potassium hydroxide and potassium nitrate, according to the method recommended by Hammarsten,† no sulphur could be found. Elastic tissue, however, unquestionably contains sulphur, but whether it exists there as a constituent part of the elastin molecule, or loosely united as in keratin, or as a constituent of some adhering proteid or other substance, it is difficult to say. Certainly, the boiling of elastic tissue with 1 per cent. potassium hydroxide for several hours might reasonably be expected to remove a part at least of any sulphur which might be present, and if by this process sulphur is removed from the tissue might it not as probably come from the elastin, as from any other proteid substance? Treatment with acid, of the alkaline solution obtained in the preparation of *A*, plainly showed the presence of hydrogen sulphide and we therefore decided in the preparation of the second portion of elastin to omit the treatment with alkali. Accordingly, 1700 grams of carefully cleaned neck bands from oxen were treated in the same manner as in *A*, except that the alkali was omitted, and in its place, treatment of the tissue with both acetic and hydrochloric acid was repeated twice. In subjecting elastin to the action of 5 per cent. hydrochloric acid care must be taken, before boiling the washed tissue with water, to see that every trace of acid is removed, otherwise the faintly acid water formed will at 100° C. give rise to a partial decomposition of the elastin.

After thorough extraction of the powdered elastin with ether, a portion dried at 110° C. gave by analysis the results shown in the following table, *Elastin B.*

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* See Zeitschrift für Biologie, Band xx, p. 11, and Amer. Chem. Jour., vol. vi, p. 3.

† Zeitschrift für physiologische Chemie, Band ix, p. 298.

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ANALYSIS OF ELASTIN B.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure. mm.	N %	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	Ash found. gram.	Ash %
I	0·5833	0·3744	7·13	1·1531	53·90	....	....	....	....	....	....	....	....
II	0·4374	0·2851	7·24	0·8653	53·94	....	....	....	....	....	....	....	....
III	0·3836	....	....	....	....	54·1	11·0	754·8	16·91	....	....	....	....
IV	0·4821	....	....	....	....	66·8	11·7	760·4	16·69	....	....	....	....
V	0·9095	....	....	....	....	....	....	....	....	0·0170	0·25	....	....
VI	0·7854	....	....	....	....	....	....	....	....	0·0208	0·36	....	....
VII	0·4235	....	....	....	....	....	....	....	....	....	....	0·0015	0·35
VIII	0·4583	....	....	....	....	....	....	....	....	....	....	0·0013	0·28

Percentage composition of ash-free substance:

							Average.
C	54·06	54·10	....	....	....	....	*54·08*
H	7·15	7·26	....	....	....	....	*7·20*
N	....	....	16·96	16·75	....	....	*16·85*
S	....	....	....	....	0·34	0·29	*0·30*
O	....	....	....	....	....	....	*21·57*
							100·00

Comparison of the two products shows a fairly close agreement in composition. In *B*, however, the carbon is slightly lower and the nitrogen a trifle higher than in *A*. Further, Elastin *B* contains 0·3 per cent. of sulphur, as determined by the modified Liebig's process.* Müller originally reported elastin, purified, however, by the use of an alkali, as containing 0·08 per cent. of sulphur, but this he considered merely as an impurity.

Morochowetz, who appears to have made a hydration product of elastin by the simple action of heat and water, states that it contains 0·617 per cent. of sulphur, but the details of his process we do not know.† If the figures are correct, his elastin must have probably contained double the percentage of sulphur present in elastin *B*. Whether pure elastin does contain sulphur, or whether the 0·3 per cent. present in preparation *B* is simply a constituent of some adhering proteid, removable by alkali, we are not at present prepared to say, but deem it probable that elastin does contain a small amount of sulphur.

* See studies from Laboratory of Physiological Chemistry, Yale University, vol. ii, p. 163.

† We have seen only an abstract in Jahresbericht für Thierchemie für 1886, p. 271.

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Elastin *A*, prepared by the usual process, shows a close agreement in composition with Horbaczewski's product, but is quite different from the product obtained by Müller and Tilanus, as seen from the following table.

	Elastin A.	*Horbaczewski.*	*Müller.**	*Tilanus.**
C	54·24	54·32	55·09–55·72	54·90–55·65
H	7·27	6·99	7·11–7·67	7·25–7·41
N	16·70	16·75	15·71–16·52	17·52–17·74

The higher content of carbon in the preparations of Müller and Tilanus are doubtless due to the presence of more or less fat, not completely extracted by ether.

Decomposition of Elastin by acid water at 100° C.

On heating moist elastin with water containing a trace of hydrochloric acid, for some hours at or near 100° C. it soon commences to swell, and after a time becomes converted into a semi-gelatinous mass, a portion of which, as the boiling continues, becomes soluble and passes into the acid fluid. The liquid gives no reaction with tannic acid for gelatin, but does give a strong biuret reaction and yields a heavy turbidity when excess of strong potassium hydroxide is added to the solution. Evidently, under these conditions, some soluble cleavage product or products are formed, percipitable by excess of caustic alkali.

These products we prepared in quantity by taking a portion of elastin *A*, soaking it for some time in 5 per cent. hydrochloric acid, until the softened elastin was thoroughly impregnated with the acid, then washing out the excess of acid by soaking it for 20 hours in a large volume of water. The elastin, still quite strongly acid to test papers, was then boiled in water for about 10 hours, when the acid liquid was strained off from the gelatinous elastin. The boiling was then continued with a fresh volume of water, the gelatinous mass still containing sufficient acid to render the whole fluid distinctly acid to test papers. This process was repeated until the elastin had been heated for 45 hours with water gradually containing less and less acid, until finally the elastin remaining had entirely lost its former gelatinous appearance and taken on its original look. All of the acid fluids were united and concentrated somewhat,

* Gorup-Besanez, Physiologische Chemie, Dritte Auflage, p. 148

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then neutralized with sodium carbonate. No neutralization precipitate was observed. By further evaporation of the neutral fluid on a water-bath, a gummy mass separated on the bottom of the dish. It was quite tenacious, something like rubber, soluble in cold water, but not so readily in hot. The cold water solution of the gummy mass, and the mother-liquor showed the following reactions:

1. Heated in a test-tube, the solution quickly became turbid, giving finally, if concentrated, a sticky precipitate. The turbidity, however, immediately disappeared as the fluid cooled, returning again when heat was applied and disappearing when cold. When the solution was so concentrated as to yield a heavy precipitate by heat, it naturally dissolved more slowly.
2. With chloroplatinic acid, a heavy yellow precipitate was formed, readily soluble in alcohol.
3. Heated with acetic acid there was no change, but addition of potassium ferrocyanide to the acid fluid caused a heavy precipitate.
4. Millon's reagent gave a strong reaction similar to the albumin reaction.
5. Strong potassium hydroxide gave a fine flocculent precipitate, insoluble in excess of the alkali.
6. Pure dilute nitric acid gave a heavy precipitate, only slowly soluble in excess of the acid. Heated, it yielded a clear yellow fluid and with ammonia gave the orange yellow color of the xanthoprotein reaction.
7. Pure dilute hydrochloric acid yielded a slight precipitate, soluble in large excess.
8. Saturation of the solution with ammonium sulphate gave a heavy, gummy precipitate.

The gummy mass and the original filtrate giving approximately the same reactions, the solution of the former was united with the latter, the fluid made slightly acid with acetic acid and then saturated in the cold with ammonium sulphate. The saturated fluid, on being boiled, gave a second gummy precipitate which was added to the first.

From analogy with the reactions of the proteoses, ammonium sulphate should precipitate any elastose bodies formed, leaving in solution the true peptone. The precipitates formed in the manner described were washed by being rubbed up in a mortar with hot saturated ammonium sulphate solution, and the washings added to the filtrate. A portion of the ammonium sulphate was then removed from the solution by concentration and crystallization, and the remainder by dialysis in running water. On testing the solution for peptone, however, none could be found; the biuret test failed to give any reaction, as did also phosphotungstic acid.

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Protoelastose.

The ammonium sulphate precipitate of elastoses, after being washed as described above, was dissolved in water, the solution made quite neutral by addition of a little sodium carbonate and then saturated with crystals of rock salt. A heavy gummy precipitate resulted, which by analogy should consist of what we may call protoelastose.

This precipitate, after being washed with saturated salt solution, was dissolved in water and reprecipitated by salt, this process being repeated three times. The final sodium chloride precipitate was dissolved in water, dialyzed until all chlorine was removed from the solution, concentrated and precipitated with 95 per cent. alcohol. Apparently, only a portion of the substance was precipitated by alcohol, so the alcoholic filtrate was evaporated and the residue carefully dried. Further examination, however, showed that both products were identical, alcohol simply precipitating a portion of the substance.

After being dried at 110° C. until of constant weight, having then the form of a brown powder, both products were analyzed with the results shown in the accompanying tables.

ANALYSIS OF PROTOELASTOSE (A1) PRECIPITATED BY ALCOHOL.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash %
						c. c.	T. °C.	Pressure. mm.			
I	0·6852	0·4271	6·92	1·3190	52·49	----	----	----	----	----	--
II	0·3926	0·2437	6·89	0·7526	52·27	----	----	----	----	----	--
III	0·5400	----	----	----	----	72·9	13·7	759·4	16·13	----	--
IV	0·3883	----	----	----	----	51·9	15·4	762·9	15·95	----	--
V	0·7048	----	----	----	----	----	----	----	----	0·0259	3·67
VI	0·5362	----	----	----	----	----	----	----	----	0·0202	3·76

Percentage composition of ash-free substance.

					Average.
C	54·51	54·28	----	----	*54·39*
H	7·19	7·16	----	----	*7·17*
N	----	----	16·75	16·56	*16·65*
O	----	----	----	----	*21·79*
					100·00

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ANALYSIS OF PROTOELASTOSE (A1) FROM THE ALCOHOLIC FILTRATE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure. mm.	N %	Ash found. gram.	Ash %
I	0·4936	0·3113	7·00	0·9626	53·18	....	....	....	....	....	..
II	0·4096	0·2592	7·03	0·8079	53·78	....	....	....	....	....	..
III	0·4588	....	....	....	....	63·2	13·4	759·8	16·49	....	..
IV	0·4267	....	....	....	....	62·5	21·6	760·8	17·06	....	..
V	0·3655	....	....	....	....	....	....	....	....	0·0054	1·47
VI	0·5096	....	....	....	....	....	....	....	....	0·0075	1·47

Percentage composition of ash-free substance.

					Average.
C	53·96	54·58	....	....	*54·27*
H	7·11	7·13	....	....	*7·12*
N	....	....	16·73	17·31	*17·02*
O	....	....	....	....	*21·59*
					100·00

The reactions of the two preparations were identical and were as follows; both were readily soluble in cold water, but only slightly soluble in hot water; in neutral solution, heat produced a turbidity which disappeared on cooling; the concentrated mineral acids gave precipitates soluble in excess; phosphotungstic acid, picric acid, tannic acid, 30 per cent. acetic acid saturated with salt, acetic acid and potassium ferrocyanide, and alcohol all produced precipitates; mercuric chloride and mercuric nitrate gave precipitates insoluble in excess; lead acetate and cupric sulphate both failed to give any precipitate; potassium mercuric iodide produced a precipitate in a hydrochloric acid solution; sodium carbonate and sodium hydroxide gave heavy precipitates and both the biuret and xanthoprotein test gave positive results. Further, the elastose is but slightly, if at all, diffusible. Long continued concentration of an aqueous solution leads to a separation of more or less of the substance as a gummy mass.

In composition, the protoelastose formed by action of the dilute acid and heat is almost identical with that of elastin itself.

Morochowetz,* in a recent paper on the laws of digestion, states that elastin by the action of heat and water passes into a new form,

* Loc. cit.

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elastose, which by further action passes into peptone. The elastose is described as a body completely soluble in water, not precipitable by mineral or acetic acids, but its solution is rendered turbid by heat. In its other reactions it resembles albumin. In composition, however, it differs decidedly from elastin and equally from our protoelastose. Morochowetz ascribes to it 55·9 per cent. carbon, 7·29 per cent. hydrogen, 16·68 per cent. nitrogen and 0·617 per cent. sulphur. As to the method employed in its preparation and purification we do not know, but as described it evidently is not akin to pure elastose.

Deuteroelastose.

In the salt-saturated filtrate from the first sodium chloride precipitate of protoelastose, there was present a substance precipitable as a gummy mass, by the addition of a little 30 per cent. acetic acid saturated with salt. Past experience with other proteoses has shown that, as a rule, all of the proto body is not precipitated by saturation of its neutral solution with salt, but that there usually remains a certain amount which on addition of salt-saturated acetic acid is precipitated, together with more or less of the deutero body, and that from this filtrate pure deutero can be separated by saturation with ammonium sulphate. In the present instance, however, it appeared that the acetic acid precipitate was of quite a different nature from the sodium chloride precipitate and that if it was not pure deuteroelastose, it at least contained only a trace of the proto body. Further, saturation of the acetic acid filtrate with ammonium sulphate gave only a slight precipitate, even when the mixture was heated, showing that if the salt-saturated acetic acid had not precipitated the deuteroelastose, little could have been formed.

The gummy precipitate separated by acetic acid and salt was purified somewhat by solution in water, and reprecipitation by saturation with salt and addition of a little salt-saturated acetic acid. It was then dissolved in water, made exactly neutral to test papers, and dialyzed until all chlorine was removed from the solution. The filtered fluid was then evaporated to dryness on a water-bath, and the powdered residue dried at 110° C. until of constant weight.

The composition of the substance is shown in the accompanying table, from which it is seen that the deutero body contains a noticeably smaller percentage of carbon than protoelastose, and a correspondingly higher content of oxygen. The difference between the sodium chloride precipitate and the acetic acid precipitate, however, becomes far more marked when the reactions of the two bodies are

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compared. Deuteroelastose was readily soluble both in cold and hot water, and on heating a cold saturated solution only a slight turbidity was seen which, as in the proto reaction, disappeared when the solution cooled. This turbidity was no doubt due to the presence of a small amount of protoelastose, for later on we obtained a purer deutero body having nearly all of the reactions of the present preparation in which, however, the heat reaction was absent.

ANALYSIS OF DEUTEROELASTOSE. A 1.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash %
						c. c.	T. ° C.	Pressure mm.			
I	0·5164	----	----	0·9894	52·24	----	----	----	----	----	----
II	0·4003	0·2499	6·93	0·7708	52·51	----	----	----	----	----	----
III	0·4648	0·2924	6·98	0·8885	52·12	----	----	----	----	----	----
IV	0·4278	0·2704	7·02	0·8149	51·94	----	----	----	----	----	----
V	0·3624	----	----	----	----	49·8	13·8	759·8	16·43	----	----
VI	0·4818	----	----	----	----	65·8	13·9	759·3	16·30	----	----
VII	0·5370	----	----	----	----	----	----	----	----	0·0105	1·95
VIII	0·4371	----	----	----	----	----	----	----	----	0·0091	2·08

Percentage composition of ash-free substance.

							Average.
C	53·30	53·57	53·19	52·99	----	----	*53·26*
H	----	7·07	7·13	7·13	----	----	*7·12*
N	----	----	----	----	16·76	16·64	*16·70*
O	----	----	----	----	----	----	*22·92*
							100·00

The deutero body, likewise, gave no precipitate whatever with the concentrated mineral acids, nor with acetic acid and potassium ferrocyanide. Alcohol gave no precipitate and salt-saturated 30 per cent. acetic acid, when added to an aqueous solution of the substance, also gave no precipitate.

Ferric chloride gave a slight turbidity, but no precipitate. With the other reactions of protoelastose, the deutero body showed close agreement. Pure deuteroelastose, formed later on by the action of pepsin-hydrochloric acid, gave no precipitate whatever with concentrated potassium hydroxide. This reaction, which is quite characteristic of protoelastose, was fairly distinct in the deutero body first described, which fact, coupled with the turbidity produced by heat, may be considered good evidence that the deuteroelastose was not perfectly pure.

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Digestion of elastin by pepsin-hydrochloric acid.

An artificial gastric juice, free from both albumose and peptone, was prepared as follows; the cardiac portions from the mucous membranes of ten pigs' stomachs (820 grams) were placed in three litres of 0·4 per cent. hydrochloric acid and warmed at 40° C. for two weeks. The clear fluid filtered from the small residue of nuclein, antialbumid, etc., was then saturated with ammonium sulphate to precipitate the pepsin. This was filtered off, washed with saturated ammonium sulphate solution to remove any adherent peptone, dissolved in two litres of 0·2 per cent. hydrochloric acid and dialyzed until the sulphate was entirely removed. The resultant solution was mixed with an equal volume of 0·4 per cent. hydrochloric acid, making a pure and active pepsin-acid mixture.

In the first digestion of elastin, 150 grams of powdered elastin *A*, 1500 c.c. of the pepsin-acid mixture, and an equal volume of 0·4 per cent. hydrochloric acid, were warmed at 40° C. for 75 hours. At the end of this time the elastin was almost entirely dissolved. The acid fluid was filtered from the small residue remaining, and neutralized with potassium hydroxide, without giving any neutralization precipitate. The reactions of the fluid showed plainly the presence of elastoses similar to those formed by the action of acid. Saturation of the fluid with ammonium sulphate gave a heavy gummy precipitate, in the filtrate from which, nothing having the reactions of peptone could be discovered.

Protoelastose.

The ammonium sulphate precipitate was dissolved in water and the neutral fluid saturated with sodium chloride, by which a heavy gummy precipitate was formed, having all of the reactions described as characteristic of protoelastose. The product was purified by several reprecipitations with salt, then dialyzed and the solution evaporated to dryness on a water-bath, the residue powdered and dried at 110° C. until of constant weight.

The composition of the substance (protoelastose A 2) is shown in the accompanying table.

In reactions, the product agrees exactly with the protoelastose already described, being precipitated by the concentrated mineral acids, by acetic acid and potassium ferrocyanide, by 30 per cent. acetic acid and sodium chloride, by strong potassium and sodium hydroxide as well as by sodium carbonate. Its aqueous solution, likewise, shows the peculiar action towards heat, already described;

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becoming turbid when heated, clear again as the solution cools. The product also gives the other reactions common to both proto- and deuteroelastose.

ANALYSIS OF PROTOELASTOSE. A 2.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %
I	0·4607	0·2880	6·94	0·9103	53·88	----	----	----	----	----	----
II	0·4952	0·3079	6·90	0·9763	53.76	----	----	----	----	----	----
III	0·5546	----	----	----	----	76·2	13·9	759·4	16·41	----	----
IV	0·3109	----	----	----	----	45·8	21·0	754·8	17·06	----	----
V	0·4603	----	----	----	----	----	----	----	----	0·0061	1·32
VI	0·5303	----	----	----	----	----	----	----	----	0·0073	1·37

Percentage composition of ash-free substance.

					Average.
C	54·58	54·46	----	----	*54·52*
H	7·03	6·99	----	----	*7·01*
N	----	----	16·64	17·29	*16·96*
O	----	----	----	----	*21·51*
					100·00

This body, which we prefer to call protoelastose, is apparently identical in composition and reactions with the *hemielastin* previously described by Horbaczewski,* and separated by him from a pepsin-acid digestion of elastin, by a method somewhat similar to the one employed by us.

At first glance, it would appear from Horbaczewski's method of separation, that his hemielastin would consist of a mixture of proto- and deuteroelastose, instead of being identical with the proto body. But the fact that he *strongly acidified* his digestive mixture with acetic acid, prior to saturating it with salt, explains the matter. Deuteroelastose, which is precipitable from a salt-saturated solution by a little acetic acid, is more or less soluble when excess of the acid is added, and hence by acidifying the mixture sufficiently, the deutero might remain dissolved while the proto would be precipitated by salt from an acid solution, equally as well as from a neutral fluid.

* Ueber das Verhalten des Elastins bei der Pepsinverdauung. Zeitschrift für physiologische Chemie, Band vi, p. 330.

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Deuteroelastose.

In the salt-saturated filtrates from protoelastose, deutero was separated as a gummy, sticky precipitate by addition of a little 30 per cent. salt-saturated acetic acid. It was purified by reprecipitation and dialysis, and the final neutral solution was evaporated to dryness and the residue dried at 110° C. until of constant weight. The amount was small and only the nitrogen was determined, 16·88 per cent.

In reactions, the body closely resembled the deutero already described; its aqueous solution giving no precipitate or turbidity whatever on the application of heat, no precipitate with alcohol, or with strong potassium hydroxide solution. It was likewise not precipitated by acetic acid and potassium ferrocyanide, by the concentrated or dilute mineral acids, nor by 30 per cent. acetic acid. In reactions, the body resembles the elastin peptone of Horbaczewski, like it being soluble in cold and warm water and dilute alcohol, and giving the same precipitations with phosphotungstic acid, picric acid, tannic acid, and with potassium mercuric iodide. It also gives the biuret and xanthoprotein reaction. It diffuses slowly.

With our present knowledge regarding peptones and proteoses, this body can hardly be considered as belonging to the former class, since it is precipitable both by ammonium sulphate, and by acetic acid when added to a salt-saturated solution; reactions not common to true peptones.

A second digestion of elastin *A* was made with the same quantities of pepsin-hydrochloric acid, and the same amount of powdered elastin, as in the first digestion. The products formed were separated in the same manner as the preceding, the elastoses being first precipitated collectively by saturation of the digestive fluid with ammonium sulphate.

On boiling the filtrate from this ammonium sulphate precipitate, a second gummy mass separated. This was collected on a cloth filter and washed with saturated ammonium sulphate solution. In the filtrate from this precipitate, no peptone could be detected. This second ammonium sulphate precipitate was purified by dialysis, etc., and the final solution evaporated to dryness and the substance dried at 110° C. Its composition is shown in the accompanying table, from which it is seen to be nearly identical with that of protoelastose. The reactions, however, indicate that it is a mixture of the two elastoses. Thus, while the concentrated mineral acids and potassium hydroxide give no precipitate, acetic acid and potassium ferrocyanide

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produce a noticeable turbidity, as does also alcohol, and acetic acid and sodium chloride. Moreover, the aqueous solution becomes somewhat clouded on heating, clearing up again as the solution cools. Hence, the reactions would indicate a preponderance of deuteroelastose, while the composition points to an excess of the proto body.

ANALYSIS OF THE SECOND AMMONIUM SULPHATE PRECIPITATE.

No	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. ° C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %
I	0·4783	0·3115	7·23	0·9404	53·61	---	---	----	----	-----	---
II	0·3781	0·2433	7·14	0·7405	53·40	---	---	----	----	-----	---
III	0·4100	-----	---	-----	----	57·7	14·2	758·0	16·76	-----	---
IV	0·3963	-----	---	-----	----	57·6	22·6	753·9	16·72	-----	---
V	0·4756	-----	---	-----	----	---	---	----	----	0·0048	1·00
VI	0·5016	-----	---	-----	----	---	---	----	----	0·0054	1·07

Percentage composition of ash-free substance.

					Average.
C	54·14	53·93	----	----	*54·03*
H	7·30	7·22	----	----	*7·26*
N	----	----	16·92	16·89	*16·90*
O	----	----	----	----	*22·81*
					100·00

We must conclude, then, that this second gummy precipitate is simply a residue of the mixed elastoses not at first precipitated, perhaps from a lack of complete saturation with ammonium sulphate. Past experience, however, has shown that deuteroproteose is not as quickly or completely precipitated with ammonium sulphate as the other proteoses.

From the first ammonium sulphate precipitate, a large amount of protoelastose (A.3) was separated, having all of the reactions characteristic of this body, and the composition, when dried at 110° C., shown in the accompanying table.

A larger amount of deuteroelastose was separated from this digestion than in the preceding one, and after purification by the use of methods already described, a portion was dried at 110° C. for analysis. Its composition is shown in the accompanying table. Like the deuteroelastose formed by dilute hydrochloric acid, it contains a lower percentage of carbon than elastin or the proto body, while its content of nitrogen is higher than that of elastin. Its reactions were the same as the deutero previously obtained.

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Deuteroelastose appears to be the same as the elastin peptone of Horbaczewski, as already mentioned; and the composition of the present product resembles it, in that it contains a similar percentage of carbon, but is unlike it in containing one per cent. more nitrogen.

ANALYSIS OF PROTOELASTOSE. A 3.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %
I	0·2990	0·1906	7·07	0·5841	53·27	---	---	----	----	-----	---
II	0·4383	0·2750	6·97	0·8606	53·54	---	---	----	----	-----	---
III	0·3938	-----	---	-----	----	57·1	19·8	760·8	16·99	-----	---
IV	0·3233	-----	---	-----	----	47·1	21·6	757·2	16·89	-----	---
V	0·5562	-----	---	-----	----	---	---	----	----	0·0081	1·45
VI	0·4866	-----	---	-----	----	---	---	----	----	0·0079	1·62

Percentage composition of ash-free substance.

					Average.
C	54·08	54·37	----	----	*54·27*
H	7·19	7·08	----	----	*7·13*
N	----	----	17·26	17·16	*17.21*
O	----	----	----	----	*21·39*
					100·00

ANALYSIS OF DEUTEROELASTOSE. A 3.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found gram.	Ash %
I	0·5067	0·3125	6·85	0·9681	52·10	---	---	----	----	----	---
II	0·6021	0·3752	6·92	1·1587	52·47	---	---	----	----	----	---
III	0·4550	0·2705	6·60	0·8685	52·05	---	---	----	----	----	---
IV	0·6886	-----	---	-----	----	96·3	13·8	759·4	16·70	----	---
V	0·3570	-----	---	-----	----	51·4	22·4	754·0	16·58	----	---
VI	0·6638	-----	---	-----	----	---	---	----	----	0·0201	3·02
VII	0·7748	-----	---	-----	----	---	---	----	----	0·0247	2·91

Percentage composition of ash-free substance.

						Average.
C	53·68	54·07	53·63	----	----	*53·79*
H	7·06	7·13	6·60	----	----	*6·99*
N	----	----	----	17·21	17·31	*17·26*
O	----	----	----	----	----	*21·96*
						100·00

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Horbaczewski's elastin peptone is described as not giving a precipitate with acetic acid and neutral salt. Deuteroelastose, likewise, gives no precipitate with acetic acid and salt, except when the solution is saturated or nearly saturated with the salt.

Another preparation of deuteroelastose was separated from a pepsin-hydrochloric acid digestion of elastin B. Its chemical composition is shown in the accompanying table, from which it is seen to differ somewhat from deutero A, but like it has a lower content of carbon than the proto body.

ANALYSIS OF DEUTEROELASTOSE *B.*

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. ° C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %
I	0·5919	0·3560	6·68	1·0934	50·37	....	....	....	....	....	....
II	0·4201	0·2572	6·80	0·7796	50·60	....	....	....	....	....	....
III	0·4852	....	....	....	....	64·9	14·0	756·8	15·91	....	....
IV	0·4719	....	....	....	....	66·0	22·4	754·2	16·11	....	....
V	0·7960	....	....	....	....	....	....	....	....	0·0398	5·00
VI	0·5882	....	....	....	....	....	....	....	....	0·0297	5·04

Percentage composition of ash-free substance.

					Average.
C	53·01	53·21	....	....	*53·11*
H	7·03	7·14	....	....	*7·08*
N	....	....	16·81	16·90	*16·85*
O	....	....	....	....	*22·96*
					100·00

Digestion of elastin with pancreatic juice.

A strong solution of pure trypsin was prepared, according to Kühne's method,* from 100 grams of dried ox pancreas and added to 150 grams of powdered elastin B in 2 litres of 0·5 per cent. sodium carbonate. The mixture was warmed at 40° C. for four or five days, a little thymol being added to prevent putrefaction. At the end of this time, the elastin was nearly all dissolved and the filtered fluid on being tested gave the ordinary elastose reactions. On saturation of a portion of the neutralized fluid with ammonium sulphate, a heavy gummy precipitate was obtained, and in the filtrate no trace of peptone reaction could be found.

* Kühne and Chittenden, Zeitschrift für Biologie, Band xix, p. 196.

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The entire digestive fluid was therefore neutralized with dilute hydrochloric acid (no neutralization precipitate), and the protoelastose at once separated by saturation with sodium chloride. The product was purified by repeated precipitation with salt, and after dialysis, the resultant fluid was evaporated to dryness and the residue dried at 110° C. for analysis. The product contained considerable ash (7·4 per cent.), consisting mainly of calcium phosphate and sulphate, with some oxide of iron. Its composition, seen in the accompanying table, is somewhat different from the protoelastose formed by pepsin-hydrochloric acid, containing as it does a noticeably lower percentage of carbon. In its reactions, however, it resembles closely the preceding preparations. It is readily soluble in cold water and the solution when heated gives the characteristic turbidity, which disappears as the solution cools. With acetic acid and potassium ferrocyanide, it gives the usual precipitate and also with the concentrated mineral acids, the latter dissolving in an excess of the acid. Unlike the protoelastose previously described, however, it gives with cupric sulphate a precipitate soluble in excess of the copper salt, and with sodium hydroxide no precipitate. In all other respects, its reactions resemble those of the proto body formed in pepsin digestion.

ANALYSIS OF THE SODIUM CHLORIDE PRECIPITATE—TRYPSIN DIGESTION OF *B.*

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %
I	0·4455	0·2635	6·57	0·8045	49·24	....	....	....	....	....	....
II	0·4650	0·2696	6·44	0·8360	49·03	....	....	....	....	....	....
III	0·3765	....	....	....	....	49·7	16·0	765·4	15·77	....	....
IV	0·6754	....	....	....	....	87·3	15·1	765·4	15·49	....	....
V	0·6473	....	....	....	....	....	....	....	....	0·0480	7·41
VI	0·8286	....	....	....	....	....	....	....	....	0·0610	7·36

Percentage composition of ash-free substance.

					Average.
C	53·15	52·95	....	....	*53·05*
H	7·09	6·95	....	....	*7·02*
N	....	....	17·02	16·74	*16·88*
O	....	....	....	....	*23·05*
					100·00

In the salt-saturated filtrate from protoelastose, the addition of a little 30 per cent. acetic acid saturated with salt gave a second

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gummy precipitate which was dissolved in water, the solution neutralized and dialyzed until all chlorine was removed. The solution, on evaporation, left a brownish residue, which was powdered and dried at 110° C. It was readily soluble in cold and hot water, its solution showing no turbidity whatever when heated. It likewise gave no precipitate with alcohol. Unlike the ordinary deutero-elastose, it did give noticeable precipitates with the mineral acids, soluble in excess, and also with acetic acid and potassium ferrocyanide. Lead acetate and cupric sulphate also gave precipitates, soluble in excess of the metallic salt. Sodium or potassium hydroxide failed to give any precipitate. In composition too, the product showed an approach to protoelastose formed by pepsin digestion, but unfortunately it contained over 5 per cent. of ash, and hence the quantitative results may perhaps be questionable.

ANALYSIS OF THE PRECIPITATE PRODUCED BY SALT-SATURATED ACETIC ACID—TRYPSIN DIGESTION OF *B.*

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash %
						c. c.	T. °C.	Pressure mm.			
I	0·4120	0·2466	6·65	0·7780	51·49	----	----	----	----	----	---
II	0·3625	0·2185	6·69	0·6899	51·89	----	----	----	----	----	---
III	0·3421	----	----	----	----	45·6	14·7	756·5	15·81	----	---
IV	0·3436	----	----	----	----	45·8	19·4	754·8	15·52	----	---
V	0·6973	----	----	----	----	----	----	----	----	0·0370	5·30
VI	0·5663	----	----	----	----	----	----	----	----	0·0337	5·60

Precentage composition of ash-free substance.

					Average.
C	54·46	54·85	----	----	*54·65*
H	7·01	7·07	----	----	*7·04*
N	----	----	16·71	16·40	*16·55*
O	----	----	----	----	*21·76*
					100·00

Of anything corresponding to heteroalbumose, we have found no trace. Further, under the conditions of our experiments, no appreciable amount of true peptone was formed in any of the digestions; at least, nothing approaching a peptone in reactions was to be found in any of the digestive fluids, after saturation with ammonium sulphate. We propose, later, to attempt a study of the elastin peptone,

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TABLE SHOWING COMPOSITION OF THE VARIOUS PRODUCTS ANALYZED.

	Elastin A.	Elastin B.	Action of acid water at 100° C.			Action of pepsin-hydrochloric acid.					Pancreatic digestion of B.	
			Protoelastose A1.		Deutero. A1.	Protoelastose.		Deuteroelastose.		A3. Second $(NH_4)_2SO_4$ P. P.	NaCl Precipitate.	NaCl + CH_3COOH Precipitate.
			Alcohol P. P.	Filtrate.		A2.	A3.	A3.	B.			
C	54·24	54·08	54·39	54·27	53·26	54·52	54·27	53·79	53·11	54·03	53·05	54·65
H	7·27	7·20	7·17	7·12	7·12	7·01	7·13	6·99	7·08	7·26	7·02	7·04
N	16·70	16·85	16·65	17·02	16·70	16·96	17·21	17·26	16·85	16·90	16·88	16·55
S	----	0·30	----	----	----	----	----	----	----	----	----	----
O	21·79	21·57	21·79	21·59	22·92	21·51	21·39	21·96	22·96	21·81	23·05	21·76
Ash	0·90	0·31	3·71	1·47	2·01	1·34	1·53	2·96	5·02	1·03	7·38	5·45

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using for this purpose the elastoses just described as well as elastin itself, and more vigorous digestive fluids, both peptic and tryptic.

In conclusion, then, we may say that elastin by the action of very dilute acid at 100° C., and by the proteolytic action of pepsin-hydrochloric acid, and of trypsin yields two primary cleavage products, which bear essentially the same relation to elastin that proto- and deuteroalbumose do to albumin. Further, that protoelastose is practically identical in reactions and composition with Horbaczewski's hemielastin.

The close relationship in composition of all the bodies analyzed is shown in the accompanying table. In considering these results, however, it is well to remember that the pancreatic preparations contain an over large amount of ash, and hence the figures may not be altogether trustworthy.

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The Influence of Urethan, Paraldehyde, Antipyrin, and Antifebrin on proteid metabolism. By R. H. Chittenden.

I. *The influence of urethan;*—from experiments made by *N. P. Washburn, Ph.B.*

Ethyl-urethan, $CO(NH_2)O.C_2H_5$, which has recently come into use as a hypnotic, is claimed by R. v. Jaksch* to be a sleep-producing agent, free from the usual disagreeable after effects common to most hypnotics. According to this observer, urethan does not appear to exert any poisonous action on the heart, nor to have any depressing influence on the arterial system. It is further assumed that the drug is without influence on digestion. Urethan acts mainly on the brain, the peripheral nervous system being unaffected, and according to v. Jaksch the hypnotic effect is always produced when the drug is taken in doses of 1 gram.

Smaller doses (0·25–0·5 gram) are uncertain in their action.

In view of the somewhat peculiar action of urethan as a hypnotic, we have undertaken to study the influence of the drug on the proteid metabolism of the healthy organism, in order to compare its action in this respect with that of other well known hypnotics.

Garnier,† alone, appears to have experimented in this direction. He states that, in the case of a man, a dose of 6 grams of urethan was followed by giddiness, etc., which condition, however, soon passed away. In this experiment, the excretion of urea was appreciably increased. In another experiment, the same observer found that a dose of 2 grams of urethan was also followed by an increased excretion of urea, in the case of a dog in a condition of hunger. A portion of the urethan was apparently converted into urea and excreted as such, but the greater part of the drug appeared in the urine unchanged. Garnier further states that large, nearly fatal doses, of the drug check

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* Abstract in Jahresbericht für Thierchemie, 1885, p. 70.

† Influence de l'urethane sur l'excretion des éléments azotés de l'urine. Compt. rend. soc. Biolog. 1886, p. 230.

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metabolism, diminishing the quantity of urine excreted, as well as the amount of urea, uric acid, etc.

The experiments about to be described were conducted wholly upon the person of a healthy man of 66 kilos. body weight. A definite amount of food, of known composition, was taken daily and uniform habits of sleep, exercise, etc., were kept up during the whole time of the experiment, which extended over a period of six weeks. In this manner, body equilibrium was established and the daily excretions brought to a constancy of composition, as preliminary to studying the action of the drug. The daily diet was as follows:

312	grams	fresh beef, free from fat and tendons.
368	"	potatoes.
227	"	wheat bread.
149	"	boiled rice.
35	"	butter.
28	"	sugar.
6	"	salt.
1200	"	water.

The body-weight was ascertained each morning, and the 24 hours' urine collected and analyzed each day as follows: nitrogen was determined by the Kjeldahl method;* sulphur by fusing a given volume of the urine with pure potassium hydroxide and potassium nitrate in a silver crucible, and ultimately precipitating and weighing the sulphur as barium sulphate;† phosphorus by fusion of a portion of the urine in a like manner with potassium hydroxide and nitrate, precipitation of the phosphoric acid from a nitric acid solution by molybdic solution, solution of this precipitate in ammonia and reprecipitation with magnesia mixture, as ammonio-magnesium phosphate; chlorine by ignition with potassium nitrate, and titration of the prepared solution with a standard solution of silver nitrate.

On April 19th, analysis of the urine was commenced and continued for fourteen days before the first dose of urethan was administered. The drug was then taken for five consecutive days, a total of 73 grains or 4·73 grams, after which the urethan was discontinued for seven days. A second trial was then made by giving 89 grains

* Neue methode zur Bestimmung des Stickstoffs in organischen Körpern. Zeitschrift für Analytische Chemie, xxii, 366.

† For the details of the processes used, see Studies from Laboratory of Physiological Chemistry, Yale University, vol. ii, p. 88.

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NORMAL URINE—WITHOUT URETHAN.

Date.	Body weight.	Reaction.	Sp. Gr.	Total volume.	Nitrogen.	Sulphur.	Phosphorus	Chlorine.	Amount of urethan taken.
April	grams			c. c.	grams	grams	grams	grams	
19	66400	acid	1027	950	17·484	1·210	1·128	5·000	0
20	66000	"	1026	1060	17·303	1·326	1·183	5·775	
21	66000	"	1028	930	16·707	1·278	1·166	4·952	
22	65800	"	1028	1010	16·891	1·160	1·141	6·191	
25	66200	"	1027	950	16·782	1·077	1·128	6·529	
26	66000	"	1023	1140	17·332	1·161	1·082	6·352	
27	65800	"	1023	1142	----	1·132	1·057	6.788	
28	65600	"	1022	1242	16·945	1·110	1·088	7·767	
29	65600	"	1023	1086	----	1·115	1·050	6·186	
30	----	"	1025	1132	----	1·264	1·195	7·850	
May									
1	65400	"	1028	1000	16·964	1·302	1·050	8·730	
2	65600	"	1028	900	17·392	1·217	1·010	5·795	
3	66000	"	1027	936	17·189	1·187	1·045	6·375	
4	65600	"	1026	1020	16·569	1·099	1·098	7·263	
Average			1025·8	1035	17·046	1·185	1·101	6·539	

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UNDER THE INFLUENCE OF URETHAN.

Date.	Body Weight.	Reaction.	Sp. Gr.	Total volume.	Nitrogen.	Sulphur.	Phosphorus	Chlorine.	Amount of urethan taken.
May	grams			c. c.	grams	grams	grams	grams	grains
5	65600	acid	1024	1188	16·161	1·163	1·184	8·459	5
6	65400	"	1018	1344	16·455	1·118	1·079	7·073	10
7	----	"	1019	1200	15·287	1·118	1·147	5·572	16
8	65200	"	1021	1165	15·568	1·184	1·473	7·068	20
9	65200	"	1029	900	16·384	1·201	1·197	6·241	22
10	65200	"	1028	930	17·704	1·214	1·056	5·528	0
11	64800	"	1028	980	17·644	1·404	1·138	6·371	
12	64600	"	1028	970	18·706	----	----	----	
13	64400	"	1021	1496	20·411	1·358	1·177	7·410	
14	----	"	1021	1254	16·258	1·306	1·146	5·512	
15	64400	"	1020	1336	18·836	1·264	1·073	7·776	
16	64200	"	1023	1182	18·680	1·272	1·090	6·367	
17	64200	"	1020	1412	18·485	1·419	1·130	8·130	20
18	63600	"	1016	1622	17·522	1·166	0·997	7·331	30
19	63200	"	1022	1130	16·597	1·265	0·977	7·707	39
20	63000	"	1026	1000	17·860	1·241	1·062	6·191	0
21	----	"	1026	950	18·397	1·269	1·093	5·558	
22	63600	"	1021	1300	21·690	1·431	1·170	7·405	
23	63600	"	1023	1116	20·192	1·442	1·156	5·873	
24	63400	"	1019	1424	20·340	1·332	1·150	7·142	

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or 5·76 grams of urethan in three consecutive days, followed by a period of six days in which the drug was not taken.

The tables, giving the amounts in grams for the 24 hours' urine of the several elements determined, show plainly that urethan has a decided action on the metabolism of the body.

On examining the results in detail, it is to be seen that urethan has a very decided diuretic action, most noticeable on the second day the drug was taken. In fact, this may be called the initial action of the drug, since in both trials the amount of water excreted, after the first increase, rapidly diminished as the dose of urethan was increased, and indeed, the volume remained far below the average amount for two or three days after the drug had been discontinued, or until its elimination from the system was fairly complete, when the volume of fluid quickly rose to normal.

The excretion of nitrogen is at once affected by urethan, even a dose of five or ten grains bringing the nitrogen noticeably below the normal amount. In both series, the excretion of nitrogen was greatly diminished. On discontinuing the drug, the nitrogen excreted rapidly increased in amount, and on the third or fourth day after its discontinuance, the daily excretion of nitrogen passed considerably above the normal.

As regards the excretion of phosphorus, it would appear from the experiments that the administration of small doses of urethan gives rise to an increased excretion of this element, as seen from the results obtained on May 5–9th. With larger doses of the drug, however, the excretion of phosphorus is diminished, as seen from the results of May 17–19th. As the excretion of sulphur runs parallel with the excretion of nitrogen, both coming from the metabolism of proteid matter, it follows that urethan when taken in small quantities must exert an inhibitory influence on proteid metabolism, while it stimulates the decomposition of certain phosphorized matters. In larger doses, the inhibitory action of the drug on proteid metabolism is still more pronounced, while at the same time the excretion of phosphorus is also retarded.

In no case was any hypnotic action noticeable.

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II. *The influence of Paraldehyde;*—from experiments made by *J. E. Dockendorff, Ph.B.*

In the following experiment, a full-blooded coach dog of 25 kilos. weight was employed. The animal was confined in a suitable cage, lined with galvanized iron and furnished with a bottom of wire netting, under which was a funnel-shaped tray, the whole so arranged as to allow all of the fluid excreta to pass into a collecting bottle underneath.

The animal was fed daily on a weighed diet consisting of dessicated beef, soda crackers and water. The beef was prepared by removing as thoroughly as possible all fat, fasciæ, tendons, etc., passing it through a sausage cutter and then drying it at a temperature below 60° C., until it had lost 75 per cent. of its weight. The dried and sampled beef was then preserved in tightly stoppered jars until needed. The crackers were ordinary soda crackers, containing about 0·7 per cent. of nitrogen. The daily rations consisted of 60 grams of crackers and 125 grams of the dessicated beef, soaked in 600 c. c. of water. This diet was commenced sometime before the urine was collected, and was continued throughout the experiment. Ultimately, the 24 hours' urine was analyzed each day, according to the methods described in the preceding experiment. Owing to irregularity of urination, and the difficulty of using a catheter, the quantity of urine obtained each day was necessarily quite variable, hence the composition of the normal urine was determined daily for three weeks, so that a sufficiently large number of results might be obtained to yield an accurate average for the normal period. Paraldehyde was then administered in gelatin capsules, about six hours after the dog had been fed, so that the drug might not interfere with digestion. Its administration was continued for eighteen days.

The results, expressed in grams for each 24 hours' urine, are shown in the accompanying tables.

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NORMAL URINE. WITHOUT PARALDEHYDE.

Date.	Total Volume.	Sp. Gr.	Reaction.	Nitrogen.	Sulphur.	Phosphorus.	Amount of Paraldehyde.
April	c. c.			grams	grams	grams	
21	395	1036	acid.	12·540	0·883	0·862	0
22	840	1025	"	19·315	1·529	1·288	
23	660	1031	"	18·276	1·176	1·442	
24	490	1035	"	14·622	1·038	1·096	
25	610	1036	"	21·050	1·388	1·340	
26	410	1036	"	13·568	----	----	
27	550	1037	"	18·550	1·396	1·202	
28	415	1036	"	13·580	1·322	0·730	
29	420	1037	"	14·916	0·940	0·934	
30	630	1038	"	18·071	1·394	1·386	
May							
1	490	1040	"	18·415	1·172	1.214	
2	390	1038	"	12·896	0·826	0·806	
3	630	1038	"	23·425	1·499	1·410	
4	525	1036	"	17·244	1·235	1·098	
5	430	1035	"	13·849	1·138	0·862	
6	375	1035	"	11·672	0·810	0·784	
7	740	1032	"	23·191	1·648	1·538	
8	465	1031	"	13·682	1·085	0·898	
9	545	1032	"	16·239	1·340	1·166	
10	535	1032	"	15·756	1·119	1·134	
11	495	1032	"	14·406	1·145	0·904	
Av'age	526	1035	acid.	16·440	1·204	1·105	

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WITH PARALDEHYDE $(C_2H_4O)_3$.

Date.	Total Volume.	Sp. Gr.	Reaction.	Nitrogen.	Sulphur.	Phosphorus.	Amount of Paraldehyde.
May	c. c.			grams	grams	grams	grams
12	540	1031	acid.	15·032	1·346	1·044	0·424
13	---	Urine	lost.	------	----	----	0·740
14	540	1028	acid.	13·624	1·000	0·776	0·715
15	310	1024	"	6·539	0·587	0·442	0·794
16	810	1027	"	20·191	1·593	1·339	0·755
17	460	1031	"	12·793	0·874	0·932	0·784
18	690	1033	"	21·091	1·444	1·296	0·702
19	525	1033	"	16·863	1·214	1·106	1·071
20	505	1032	"	14·523	1·016	0·978	1·536
21	735	1029	"	20·844	1·475	1·292	1·499
22	545	1031	"	16·756	1·122	0·462	1·811
23	555	1031	"	17·063	1·195	1·620	2·268
24	580	1032	"	18·202	1·245	1·080	2·675
25	360	1030	"	9·935	0·734	0·590	3·111
26	800	1033	"	25·695	1·924	1·476	3·632
27	460	1030	"	12·992	0·935	0·766	4·063
28	585	1033	"	19·174	1·368	0·990	4·958
29	695	1032	"	21·756	1·391	1·342	5·941
Av'age	570	1030	acid.	16·060	1·203	1·031	37·479

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WITHOUT PARALDEHYDE.

Date.	Total Volume.	Sp. Gr.	Reaction.	Nitrogen.	Sulphur.	Phosphorus.	Amount of Paraldehyde.
May	c. c.			grams	grams	grams	
30	610	1028	acid.	17·672	1·309	0·992	0
31	570	1031	"	17·080	1·311	1·154	
June 1	440	1028	"	12·201	0·765	0·780	
2	645	1031	"	19·550	1·376	1·299	
3	640	1030	"	17·798	1·237	1·608	
4	445	1026	"	11·112	0·979	....	
Av'age	558	1029	acid.	15·902	1·163	1·166	

Throughout the experiment, the dog appeared perfectly well, and at no time showed any symptoms of nausea. Neither was there any special hypnotic action noticeable.

The average of the results shows plainly that the drug has little, if any, action on proteid metabolism. Under the influence of the paraldehyde there was a slight increase in the amount of water excreted. Bokai * has stated that the urinary secretion is slightly increased by paraldehyde. In our experiment, however, the diuretic action is not great. As regards the excretion of nitrogen, there is a slight diminution to be seen in the paraldehyde period. There is also a corresponding decrease in the excretion of phosphorus. The three periods, however, show such close agreement in results, it is obvious that, under the conditions of this experiment, paraldehyde has not exerted any special influence on proteid metabolism.

According to the experiments of Quinquad and A. Hénocque,† paraldehyde causes a diminution of body temperature, and at the same time a very noticeable falling off in the excretion of carbonic acid; thus, according to one of Quinquad's experiments, a dog after receiving by hypodermic injection 8 c. c. of paraldehyde gave off 5·5 grams of carbonic acid, while it expired during the same time, three-fourths of an hour after the injection, only 1·96 grams of carbonic acid.

* Ueber die physiologische Wirkung des Paraldehyds. Centralblatt für die medicinische Wissenschaften. 1887, p. 412.

† Abstract in Jahresbericht für Thierchemie. 1884, p. 374.

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III. *Influence of Antipyrin*;—from experiments made by *H. F. Adams, Ph.B.*

Previous experiments* with antipyrin, made in this laboratory, have shown that this drug, when introduced into the stomach of healthy rabbits, has little, if any, noticeable influence on the excretion of carbonic acid or on the body temperature, except in the case of toxic doses. Coppola,† likewise, found that the subcutaneous introduction of 0·1 to 0·3 gram of antipyrin in the case of dogs led to a reduction of body temperature of only 0·25 to 0·6 of a degree, while 0·3–0·4 gram of the drug was without any noticeable influence upon the excretion of urea. In fever patients, F. Müller,‡ however, had previously noticed a diminution in the excretion of nitrogen under the influence of antipyrin, while in the case of healthy men the excretion was affected but very slightly, if at all. Jacubowitsch,§ likewise, had noticed in experiments on healthy and fevered children that the use of antipyrin led to a diminution both in the quantity and specific gravity of the urine, and also a diminution in the quantity of uric acid, phosphoric acid, sulphuric acid and of chlorides. L. Riess,‖ by carefully conducted experiments on nine typhus fever patients, found as a principal result that antipyrin in doses up to 12 grams per day diminished considerably the excretion of nitrogen, the diminution ranging in six series of experiments from 2·5 to 24·7 per cent. Umbach,¶ likewise, has studied the influence of antipyrin on the excretion of nitrogen, both on a dog and on himself, and he found that with a definite diet the excretion of nitrogen sank, under the influence of 4 grams of antipyrin, in two days about 2 grams, equal to 4 grams of urea. The uric acid excretion, however, was not materially affected.

In spite of these manifold experiments, we have deemed the matter of sufficient importance to warrant further study, especially with a view to the action of the drug on the metamorphism of nitrogenous matter in the *healthy* organism. The experiments were therefore tried upon a healthy man with a body weight of 77 kilograms, and

* Chittenden and Cummins. Studies, vol. ii., p. 231.

† Abstract in Jahresbericht für Thierchemie. 1885, p. 98.

‡ Abstract in Jahresbericht für Thierchemie. 1884, p. 242.

§ Abstract in Jahresbericht für Thierchemie. 1885, p. 444.

‖ Archiv für experim. Pathol. u. Pharm. xxii, 127; also Abstract in Jahresbericht für Thierchemie. 1886, p. 417. Ueber stickstoffausscheidung bei antipyretischer Fieberbehandlung.

¶ Ueber den Einfluss der Antipyrins auf die stickstoffausscheidung. Abstract in Jahresbericht für Thierchemie. 1886, p. 418.

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under definite conditions of diet, exercise, etc. Nitrogenous equilibrium was established prior to the experiment, and the following daily diet was strictly adhered to throughout the entire period.

386	grams	meat (beef).
340	"	potatoes.
227	"	wheat bread.
168	"	oat meal (steamed).
28	"	sugar.
42	"	butter.
120	"	milk.
1040	"	water.

In this experiment, nitrogenous metabolism was measured by determining the urea and uric acid contained in the daily excretion, instead of determining the total nitrogen. This was done in order to ascertain whether the drug has any special action on the excretion of uric acid. Urea was determined by Liebig's method, as modified by Pflüger.* Chlorine was previously determined by fusion with potassium nitrate and titration with silver nitrate in the usual manner, and then removed from the solution to be tested for urea, by a standard silver solution. Uric acid was determined by Salkowski's method, and phosphoric acid by titration with a standard uranium solution.

The accompanying tables give the results of the daily analyses. From these it is evident that under the conditions of this experiment, antipyrin has a decided inhibitory action on the proteid metabolism of the healthy human organism, as shown by the diminished excretion of urea and uric acid when the drug is taken. Antipyrin also tends to diminish the volume of the urinary secretion, this action being very marked in the second series, where comparatively large amounts of antipyrin were administered. As regards the excretion of phosphoric acid and of chlorine, nothing definite can be said. The more important changes produced by the antipyrin are shown in the following table of average daily results.

	Urea. grams.	Uric acid. gram.	Total P_2O_5. grams.	Volume. c. c.	Sp.Gr.
Normal period	41·806	0·586	3·185	951	1028
First antipyrin period	38·375	0·556	3·026	848	1029
First after period	42·030	0·575	2·929	929	1028
Second antipyrin period	40·854	0·472	2·941	822	1031
Second after period	44·220	0·537	2·923	957	1028

* Pflüger's Archiv für Physiologie, vol. xxi, p. 248.

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NORMAL URINE.—WITHOUT ANTIPYRIN.

Date.	Body weight.	Reaction.	Sp. Gr.	Total volume.	Chlorine.	Total P_2O_5.	P_2O_5 with Ca and Mg.	Uric acid.	Urea.	Amount of Antipyrin taken.
April.	grams			c. c.	grams	grams	gram	gram	grams	
24	77000	acid	1030	840	4·660	3·264	0·492	0·543	42·359	0
25	77200	"	1028	970	6·557	3·126	0·524	0·595	42·120	
26	77200	"	1027	950	3·820	3·236	0·499	0·570	41·427	
27	77200	"	1025	1085	5·407	3·043	0·509	----	41·026	
28	77000	"	1027	1040	6·113	3·341	0·541	0·702	42·768	
29	77000	"	1026	1090	5·781	2·957	0·561	0·651	41·616	
30	76900	"	1027	1000	6·006	3·284	0·601	0·636	41·492	
May										
1	76800	"	1029	970	6·756	3·166	0·586	0·560	41·861	
2	76800	"	1030	870	4·113	3·400	0·483	0·542	42·141	
3	76800	"	1030	860	4.117	3·238	0·495	0·571	41 913	
4	76600	"	1031	790	3·279	2·980	0·483	0·491	41·143	
Average			1028	951	5·146	3·185	0·525	0·586	41·806	

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UNDER THE INFLUENCE OF ANTIPYRIN.

Date.	Body weight.	Reaction.	Sp. Gr.	Total volume.	Chlorine.	Total P_2O_5.	P_2O_5 with Ca and Mg.	Uric acid.	Urea.	Amount of Antipyrin taken.
May	grams			c. c.	grams	grams	gram	gram	grams	grains
5	76600	acid	1031	800	3·016	3·288	0·599	0·501	40·995	30
6	76600	"	1031	780	3·688	3·118	0·596	0·548	38·391	30
7	76700	"	1027	1050	5·009	2·559	0·535	0·625	38·060	30
8	76800	"	1029	800	4·089	3·059	0·610	0·548	37·168	45
9	76600	"	1030	810	3·985	3·106	0·603	0·557	37·260	54
Average			1029	848	3·975	3·026	0·588	0·556	3..375	
10	76800	"	1023	900	4·658	2·652	0·550	0·607	39·744	0
11	76400	"	1026	890	3·867	2·541	0·567	0·602	42·895	
12	76400	"	1028	910	4·203	2·821	0·555	0·602	41·860	
13	75800	"	1028	1000	4·281	3·009	0·535	0·498	43·080	
14	75800	"	1028	915	3·625	3·265	0·589	0·582	42·510	
15	75800	"	1028	910	4·535	3·202	0·598	0·642	42·110	
16	75600	"	1028	980	4·759	3·011	0·514	0·495	42·012	
Average			1028	929	4·275	2·929	0·558	0·575	42·030	

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UNDER THE INFLUENCE OF ANTIPYRIN—*Continued.*

Date.	Body weight.	Reaction.	Sp. Gr.	Total volume.	Chlorine.	Total P_2O_5.	P_2O_5 with Ca and Mg.	Uric acid.	Urea	Amount of Antipyrin taken.
May	grams			c. c.	grams	grams	gram	gram	grams	grains
17	75400	acid	1030	880	4·442	3·286	0·542	0·534	41·694	40
18	75600	"	1030	880	4·048	3·335	0·627	0·564	42·404	50
19	75800	"	1032	790	4·341	3·052	0·635	0·406	41·143	60
20	74600	"	1032	760	4·273	2·748	0·588	0·385	39·227	60
21	.....	"	1032	800	4·805	2·284	0·574	0·472	39·804	60
Average			1031	822	4·382	2·941	0·593	0·472	40·854	
22	75400	"	1030	910	4·477	2·652	0·590	0·621	44·076	0
23	74800	"	1028	990	3·795	2·632	0·500	0·516	45·114	
24	74800	"	1027	970	4·834	2·945	0·495	0·533	43·751	
25	74800	"	1027	965	3·576	3·196	0·532	0·514	45·490	
26	74600	"	1029	950	3·885	3·190	0·520	0·504	42·673	
Average			1028	957	4·113	2·923	0·527	0·537	44·220	

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Since this work was finished we have seen an interesting paper by Dr. Kumagawa* on the action of certain antipyretics on proteid metabolism, in which is given the results of an experiment with antipyrin on the excretion of nitrogen and uric acid, in the case of a dog of 26 kilos. weight in a condition of nitrogenous equilibrium.

In this experiment, Dr. Kumagawa found that even large doses of antipyrin (51 grams in 16 days) produced no change whatever in the excretion of nitrogen (determined by the Kjeldahl method), but that there was a very noticeable increase in the excretion of uric acid (determined by Salkowski's method), amounting on an average to 65 per cent. above the normal excretion. These results stand in direct opposition to what we have found with somewhat smaller doses, in experimenting on the human organism. Whether the explanation of this difference is to be found in the different nature of the two organisms experimented with we cannot now say, but we hope at a later date to explain this apparently divergent action.

IV. *The influence of antifebrin*;—from experiments made by *H. C. Taylor, Ph.B.*

Antifebrin or acetanilide, which has recently come into use as an antipyretic, as a nervine and antiseptic, has been the subject of many clinical observations but has not as yet, so far as we know, been experimented with to ascertain its influence on proteid metabolism. We have endeavored, therefore, to ascertain the influence of this new antipyretic on the nutrition of the healthy human organism, believing that such results may possibly be of greater value than those obtained by experimenting on animals. At the same time it is to be borne in mind, that an antipyretic especially may produce an effect upon the healthy organism quite different from that which the same doses would produce on an organism rendered perhaps more susceptible by disease, as in fever. The experiment was therefore conducted upon the person of a young man of 64 kilos. body weight, brought into a condition of nitrogenous equilibrium and maintained throughout the experiment upon a weighed diet of known composition. For reasons already given, the excretion of nitrogenous matter was measured by determining in the 24 hours' urine the amount of urea and uric acid, using the methods employed in one of the preceding experiments. Sulphur, phosphorus and chlorine were also

*Ueber die Wirkung einiger antipyretischer Mittel auf den Eiweissumsatz im Organismus. Virchow's Archiv, Band cxiii, p. 192.

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determined each day by methods already indicated. After nitrogenous equilibrium had been established, and the urine analyzed for ten consecutive days, antifebrin was administered daily in divided doses, at a time not to interfere with digestion, for a period of nine days. The daily dose was gradually increased until at last there was a slight approach to cyanosis. In all, 13·3 grams or 205 grains of the antipyretic were taken. No disagreeable symptoms were experienced, but there was a very noticeable lividity of countenance with a decided blueness of the lips, and a slight darkening of the skin near the cheek bones.

The initial daily dose was 0·4 of a gram or a little over 6 grains, and was rapidly increased to 2·6 grams or 40 grains per day, given in three doses. There are, to be sure, many cases recorded where apparently smaller doses have led to serious results, but careful watching failed to show any symptoms whatever even suggestive of any disagreeable action on the digestive system, the circulation or respiration. Weinstein, indeed, has said that persons not suffering from fever may take antifebrin for weeks together without any effect on the circulation, while according to Herczel the long-continued administration of antifebrin, thirty to forty-five grains daily for six weeks, may lead to what he terms aniline anaemia with solution and decomposition of the hæmoglobin of the blood. In fact, the latter observer considers that aniline is set free from the acetanilide and that the decomposition of the blood-coloring matter is due to this cause. Whether this is the cause of the cyanosis so often spoken of in connection with this drug is uncertain.

In the second antifebrin period, 13·9 grams or 214 grains of the drug were taken in seven days, accompanied at the close with the same approach to cyanosis as before.

Examination of the analytical results shows plainly that under the conditions of this experiment the excretion of urea is not very greatly affected. There is, however, in both antifebrin periods a slight increase, indicating increased proteid metabolism under the influence of the drug. This increased excretion of urea is more apparent in the individual results than in the average of the series. Thus, in the normal urine it is to be noticed that the daily excretion of urea never exceeded 34·5 grams, while in the first antifebrin period, on the days when the largest doses of acetanilide were taken, the excretion of urea amounted to 35–37 grams, and in the after period quickly fell to about 33 grams per day. The same peculiarity is also noticeable, to a less extent, in the second antifebrin period.

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NORMAL URINE—WITHOUT ANTIFEBRIN.

Date.	Body weight.	Reaction.	Sp. Gr.	Total volume.	Urea.	Uric acid.	Sulphur.	Phosphorus.	Chlorine.	Amount of Antifebrin.
April	grams			c. c.	grams	gram	grams	grams	grams	
23	64200	acid	1025	790	33·331	----	1·380	0·966	2·967	0
24	64200	"	1016	1280	33·280	0·749	1·154	0·864	4·404	
25	64000	"	1017	1200	33·486	0·708	1·207	1·227	3·603	
26	64000	"	1014	1340	33·947	0·739	1·254	0·859	3·520	
27	64000	"	1018	1030	33·058	0·753	1·220	1·041	3·221	
28	64000	"	1017	1150	34·502	0·779	1·296	0·881	4·027	
29	63800	"	1015	1250	32·619	0·723	1·222	0·862	4·377	
30	64200	"	1023	880	33·440	0·741	1·289	0.842	4·073	
May										
1	64000	"	1013	1590	34·370	0·694	1·350	0·821	6·366	
2	64000	"	1016	1250	33·334	0·777	1·124	1·122	4·078	
Average			1017	1176	33·536	0·740	1·249	0·948	4·063	

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FIRST ANTIFEBRIN PERIOD.

Date.	Body weight.	Reaction.	Sp. Gr.	Total volume.	Urea.	Uric acid.	Sulphur.	Phosphorus.	Chlorine.	Amount of Antifebrin.
May	grams			c. c.	grams	grams	grams	grams	grams	grams
3	64000	acid	1015	1400	33·190	0·671	1·241	0·932	4·743	0·4
4	64000	"	1013	1630	34·155	0·762	1·313	0·910	5·505	0·7
5	64000	"	1017	1190	29·920	0·611	1·096	0·510	4·912	0·9
6	64000	"	1014	1770	35·063	0·670	1·380	1·167	6·533	1·2
7	64000	"	1018	1190	29·843	0·538	1·241	0·992	4·153	1·5
8	64000	"	1017	1300	33·882	0·651	1·353	1·012	0·520	2·4
9	63800	"	1017	1230	35·172	0·572	1·294	0·948	4·886	2·6
10	63800	"	1017	1280	35·996	0·639	1·299	0·846	3·843	2·6
11	63800	"	1015	1470	37·011	0·596	1·318	1·102	4·782	1·0
Average			1016	1384	33.804	0·634	1·282	0·935	4·375	

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FIRST PERIOD AFTER ANTIFEBRIN.

Date.	Body weight.	Reaction.	Sp. Gr.	Total volume.	Urea.	Uric acid.	Sulphur.	Phosphorus.	Chlorine.	Amount of Antifebrin.
May	grams			c. c.	grams	grams	grams	grams	grams	
12	63800	acid	1017	1210	32·965	0·690	1·372	0·858	4·314	0
13	63800	"	1013	1800	33·596	0·705	1·374	0·982	5·405	
14	63600	"	1016	1400	33·596	0·650	1·412	0·965	4·292	
15	63600	"	1015	1560	36·245	0·715	1·214	1·099	4·465	
16	63600	"	1013	1830	34·799	0·785	1·489	1·089	5·380	
17	63400	"	1016	1370	33·437	0·755	1·443	0·883	4·484	
18	63400	"	1016	1400	33·904	0·755	1·356	0·878	5·904	
19	63500	"	1013	1710	32·736	0·640	1·588	0·969	5·472	
20	63600	"	1016	1500	32·377	0·645	1·376	0·980	6·043	
Average			1015	1531	33·739	0·704	1·404	0·967	5·084	

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SECOND ANTIFEBRIN PERIOD.

Date.	Body weight.	Reaction.	Sp. Gr.	Total volume.	Urea.	Uric acid.	Sulphur.	Phosphorus.	Chlorine.	Amount of Antifebrin.
May	grams			c. c.	grams	grams	grams	grams	grams	grams
21	63800	acid	1017	1280	34·027	0·437	1·360	0·959	5·814	1·0
22	63800	"	1016	1340	33·673	0·460	1·312	0·841	5·314	2·0
23	63800	"	1019	1270	33·882	0·592	1·272	0·860	5·995	2·2
24	63800	"	1018	1340	33·968	0·570	1·235	0·876	7·001	2·5
25	63800	"	1018	1420	34·579	0·595	1·432	0·874	8·302	2·5
26	63800	"	1022	1220	32·990	0·626	1·249	0·996	6·295	2·5
27	63600	"	1018	1510	34·765	0·721	1·399	0·864	7·510	1·2
Average			1018	1340	33·983	0·571	1·323	0·896	6·604	
28	63600	"	1014	1800	33·792	0·596	1·492	1·017	8·158	0
29	63600	"	1022	1000	33·201	0·639	1·375	0·785	5·917	
30	63600	"	1025	920	32·880	0·681	....	0·876	5·279	
Average			1020	1240	33·291	0·639	1·423	0·892	6·451	

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We must conclude, therefore, that acetanilide tends to increase somewhat the excretion of urea, but that with such doses as we have employed the increase in proteid metabolism cannot be great. This is further indicated by the lack of any corresponding change in the excretion of sulphur.

The excretion of phosphorus is also unaffected by antifebrin.

On the excretion of uric acid, however, our results indicate a special inhibitory influence. This is quite apparent both in the averages of the different series and in the individual results, and, if correct, would appear to be the most marked characteristic of antifebrin, so far as its influence on proteid metabolism is concerned. Various observers have stated that antifebrin acts as a diuretic, others that it decreases the secretion of water, and while doubtless both results have been seen to follow its administration in diseased conditions of the system, our experiment on a healthy man gives no evidence of any action of this kind.

Since the foregoing was written we have seen the results of Kumagawa's* experiment with antifebrin on a dog, from which he concludes that acetanilide taken in small doses (2–3 grams per day) does not give rise to any appreciable increase in the decomposition of proteid matter, but that larger doses (4–5 grams per day) cause a very marked increase, as indicated by the increased excretion of nitrogen. Such doses are, however, as Kumagawa himself admits, too large, especially when given to a moderate sized dog, to have the results of any practical value. Lépine,† too, experimenting on a hungry dog, thought he found an increase in the excretion of nitrogen after giving two doses of one and two grams of antifebrin. Further, Ademski‡ is quoted as considering that urea is increased, but the total quantity of nitrogen decreased by antifebrin. Bokai, that the amount of nitrogen is diminished and Berezooski that the urea decreases with the fall of temperature. Whether these latter views are founded on actual experiments or are mere conjecture I do not know.

According to Jaffe and Hilbert,§ rabbits fed upon antifebrin excrete it mainly as paramidophenol-sulphuric acid, and Kumagawa has likewise found, in the case of a dog, that neither acetanilide nor aniline appear in the urine, but that the antifebrin is excreted mainly as paramidophenol united to sulphuric acid.

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* Virchow's Archiv. Band cxiii, p. 171.

† See Salkowski's Bemerkung in Virchow's Archiv, Band cxiii, p. 394.

‡ See Report on Antifebrin in the Therapeutic Gazette, vol. xii, p. 571.

§ Zeitschrift für physiologische chemie, xii, p. 307.

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IV.—The influence of several new therapeutic agents on amylolytic and proteolytic action. By R. H. Chittenden and C. W. Stewart, Ph.B.

In view of the pronounced action of a number of newly discovered therapeutic agents on metabolism, we have deemed it of importance to widen our knowledge regarding their physiological action by attempting a study of their behavior towards the amylolytic and proteolytic ferments, with the hope of gaining some insight into their influence on normal digestion.

The methods employed were similar to those used in previous experiments of this kind,* in which the action of varying percentages of the drug were determined quantitatively.

Influence on amylolytic action.

As amylolytic ferment, human mixed saliva was employed, filtered and carefully neutralized, and then diluted with distilled water in the proportion of 1 to 5. The experiments were made in series, in which one digestion of each series served as a control for comparison. The volume of each digestive mixture was 100 c. c., in which was present 1 gram of perfectly neutral potato starch previously boiled with a portion of the water, 10 c. c. of diluted neutral saliva and a given quantity of the substance to be experimented with. The mixtures were warmed at 40° C. for thirty minutes, after which further action of the ferment was stopped by heating the solution to boiling. The extent of amylolytic action was then ascertained, by determining in one-fourth of the solution the amount of reducing substances by Allihn's gravimetric method.† For the sake of convenience, the total amount of reducing substance was calculated as dextrose, from which in turn was calculated the percentage of starch converted.

Antipyrin.

With this new antipyretic, several series of experiments with small percentages were made which show clearly that the substance is

* Studies from the Laboratory of Physiological Chemistry, Yale University, vols. i and ii.

† Zeitschrift für analytische chemie, xxii, p. 448.

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without any appreciable influence. When present in larger percentages, the drug was found to have a slight inhibitory influence on amylolytic action, as the following series of experiments show :

Per cent. of Antipyrin.	Total amount of reducing substances.	Starch converted.	Relative amylolytic action.
0	0·3446 gram.	31·01 per cent.	100·0
0·5	0·3446	31·01	100·0
1·0	0·3424	30·82	99·4
3·0	0·3278	29·50	95·1
5·0	0·3112	28·01	93·6

Coppola,* in studying the physiological action of antipyrin, found that three per cent. of the substance did not hinder the inversion of cane sugar by yeast, but did prevent alcoholic fermentation. Further, that the same percentage hindered slightly the action of malt diastase on starch, and had a decided inhibitory influence on the alkaline fermentation of urine.

Antifebrin.

Owing to the comparative insolubility of antifebrin or acetanilide in water, large percentages could not be employed. Such as were used, however, clearly show that this antipyretic has little influence on amylolytic action.

Per cent. of Antifebrin.	Total amount of reducing substances.	Starch converted.	Relative amylolytic action.
0	0·3604 gram.	32·44 per cent.	100·0
0·10	0·3600	32·40	99·9
0·25	0·3638	32·70	100·8
0·50	0·3550	32·00	98·6
0	0·3382	30·44	100·0
2·0	0·3298	29·68	97·5

In this connection, it is interesting to notice that Kumagawa* has found antifebrin to have a strong antiseptic action on the putrefactive processes of the intestinal canal, and Van Seer has observed that milk does not undergo fermentation if saturated with it, and also that it will prevent albumin becoming putrid.†

Urethan.

With ethyl-urethan, two series of experiments were tried, with the following results :

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* Jahresbericht für Thierchemie. 1885, p. 98.

† Virchow's Archiv, Band cxiii, p. 184.

‡ See The Therapeutic Gazette, vol. xii, p. 566.

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Per cent. of Urethan.	Total amount of reducing substances.	Starch converted.	Relative amylolytic action.
0	0·3382 gram.	30·44 per cent.	100·0
0·5	0·3550	31·95	104·6
0	0·3402	30·62	100·0
2·0	0·3446	31·01	101·3
3·0	0·3352	30·17	98·5
5·0	0·3268	29·40	96·0

Here, there is noticeable, with the smaller percentages, a slight stimulation of amylolytic action, but it is not sufficiently large to be very marked.

Paraldehyde.

This substance shows very strong inhibitory action, even 0·5 per cent. diminishing the amount of starch converted by 30 per cent. It must be remembered, however, that the aldehyde is only slightly soluble in water, and that it is more or less volatile. The experiments were conducted in small flasks and the aldehyde kept more or less emulsionized with the aqueous solution by shaking the mixtures, but obviously the percentages given can only approximately represent the amount actually taking part in the reaction.

Following are the results of two series of experiments:

Per cent. of Paraldehyde.	Total amount of reducing substances.	Starch converted.	Relative amylolytic action.
0	0·3554 gram.	31·98 per cent.	100·0
0·1	0·3534	31·81	99·5
0·2	0·3528	31·75	99·3
0·5	0·2468	22·21	69·5
1·0	0·1066	9·59	30·0
0	0·3554	31·98	100·0
0·8	0·1392	12·52	39·1
1·0	0·0948	8·53	26·6
1·5	0·0620	5·58	14·3
2·0	0·0446	4·01	12·5

Thallin Sulphate.

This salt has a more marked influence on the amylolytic action of saliva than any of the preceding substances. Very small percentages have a noticeable stimulating action, while the presence of 0·2 per cent. of the salt almost entirely stops the action of the ferment. The results of the following two series show one or two small points of difference, but in the main they point to the same general action.

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Per cent. of Thallin Sulphate.	Total amount of reducing substances.	Starch converted.	Relative amylolytic action.
0	0·3534 gram.	31·81 per cent.	100·0
0·025	0·3648	32·83	103·2
0·050	0·3810	34·36	108·0
0·100	0·3540	31·86	100·1
0·200	0·0690	6·21	19·5
0·300	trace.		
0	0·3652	32·86	100·0
0·025	0·3798	34·18	104·0
0·050	0·3872	34·89	106·1
0·080	0·3812	34·81	105·9
0·10	0·3148	28·33	86·2

Caffein and Thein.

These two closely related alkaloids were found to have only a slight action on the amylolytic ferment, producing when present in considerable amount a slight diminution in the amount of starch converted. With two per cent. of the alkaloids, the following results were obtained:

Per cent. of alkaloid.	Total amount of reducing substances.	Starch converted.	Relative amylolytic action.
0	0·3620 gram.	32·57 per cent.	100·0
2·0 Caffein.	0·3342	30·08	92·4
0	0·3664	32·97	100·0
0·2 Thein.	0·3440	30·96	93·9

Influence on proteolytic action.

The influence of the above therapeutic agents on the proteolytic action of pepsin-hydrochloric acid was determined, as in preceding work of this kind,* by ascertaining the amount of fibrin digested or dissolved in a given time, by a definite volume of standard, artificial gastric juice, in the presence of varying amounts of the substances to be tested. The gastric juice was made by dissolving 10 c. c. of a glycerin extract of pepsin in one litre of 0·2 per cent. hydrochloric acid. The volume of each digestive mixture was 50 c. c., composed of 25 c. c. of the above mentioned artificial gastric juice, and 25 c. c. of 0·2 per cent. hydrochloric acid containing the desired amounts of the substances to be tested. The proteid material consisted of purified fibrin, coarsely powdered and dried at 110° C. One gram of fibrin was used in each experiment. The digestive mixtures were warmed at 40° C. for a certain length of time, usually two hours,

* Studies from the Laboratory of Physiological Chemistry of Yale University, vols. i and ii.

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after which further proteolytic action was stopped by heating the mixtures to boiling. The undissolved residues were then collected on dried, weighed filters, washed thoroughly with boiling water, and finally dried at 110° C. until of constant weight. The amount of fibrin dissolved is taken as a measure of proteolytic action.

Following are the results obtained with the various substances tested:

Antipyrin.

Per cent. of Antipyrin.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·507 gram.	49·3 per cent.	100·0
0·2	0·522	47·8	96·9
0·5	0·573	42·7	86·6
1·0	0·688	31·2	63·3
	Longer time at 40° C.		
0	0·143	85·7	100·0
0·5	0·213	78·7	91·8
3·0	0·964	3·6	4·2
5·0	1·008	0	0

Antifebrin.

Per cent. of Antifebrin.	Undigested residue.	Fibrin digested.	Relative proteolytic action
0	0·129 gram.	87·1 per cent.	100·0
0·1	0·145	85·5	98·1
0·2	0·166	83·4	95·8
0·5	0·212	78·8	90·5
1·5	0·371	62·9	72·2

Urethan.

Per cent. of Urethan.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·148 gram.	85·2 per cent.	100·0
0 2	0·138	86·2	101·2
1·0	0·171	82·9	97·2
3·0	0·224	77·6	90·9
5·0	0·240	76·0	89·4

Paraldehyde.

Per cent. of Paraldehyde.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·263 gram.	73·7 per cent.	100·0
0·05	0·219	78·1	105·9
0·10	0·246	75·4	102·3
0·30	0·255	74·5	101·1
1·00	0·264	73·6	99·9
2·00	0·269	73·1	99·2

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Thallin sulphate.

Per cent. of Thallin sulphate.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·379 gram.	62·1 per cent.	100·0
0·05	0·243	75·7	121·9
0·10	0·327	67·3	108·4
0·30	0·395	60·5	97·4

Thein and Caffein.

Per cent. of alkaloid.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·105 gram.	89·5 per cent.	100·0
1·0 Thein.	0·252	74·8	84·7
4·0 "	0·179	82·1	91·8
4·0 Caffein.	0·584	41·6	46·5

These results show that antipyrin has a decided inhibitory influence on the action of the proteolytic ferment, and that when present to the extent of 3·0 per cent. it practically stops all digestive action. Antifebrin has also an inhibitory action, perhaps equal to that of antipyrin, but owing to its greater insolubility in acid fluids large percentages of the substance could not be tested.

Urethan has only a slight retarding action, even when present to the extent of 5 per cent.

Paraldehyde and thallin sulphate both show a very decided stimulating action when small fractions of one per cent. are present, the latter, particularly, causing a much larger amount of fibrin to be digested than in the control experiment.

Thein and caffein both show an inhibitory influence on the ferment, that of caffein being much greater than that of thein.

On the proteolytic action of trypsin in an alkaline solution, two substances only were tested, antifebrin and paraldehyde. The experiments were conducted in the same manner as with pepsin-hydrochloric acid, except that a solution of trypsin in 0·3 per cent. sodium carbonate was employed in place of the pepsin-acid, and the mixture warmed for a longer time at 40° C. Following are the results obtained, showing a much more pronounced inhibitory action on this ferment than on pepsin.

Per cent. of substance.	Undigested residue.	Fibrin digested.	Relative proteolytic action.
0	0·159 gram.	84·1 per cent.	100·0
0·2 antifebrin.	0·368	63·2	75·1
1·0	0·741	25·9	30·8
0·2 paraldehyde.	0·368	63·2	75·1
2·0	0·959	4·1	4·9

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V.—Caseoses, Casein Dyspeptone, and Casein Peptone. By R. H. Chittenden.

In a previous paper on "Casein and its Primary Cleavage Products,"* the writer expressed the intention of continuing the study of the caseoses formed in pepsin digestion, and also of studying pure casein peptone and the so-called casein dyspeptone. In the fulfillment of this intention, experiments upon these subjects have been carried on in this laboratory during the past two years, with the aid of several co-workers, and the results are here presented collectively.

I.—*Casein Dyspeptone*;—from experiments by *L. A. Conner, Ph.B.*, and *C. A. Tuttle, Ph.B.*

When casein is exposed to the action of pepsin-hydrochloric acid at 40° C., or even at ordinary temperatures, it is decomposed, as is well known, into soluble caseoses and peptone. In every such digestion, however, there always remains a certain amount of an insoluble pasty, grayish white substance, which apparently is not susceptible to the further action of gastric juice, no matter how long continued. This insoluble substance, which is noticeable to a greater or less extent in the pepsin digestion of all proteids, but particularly so with casein, and which received from Meissner the name of dyspeptone, was examined somewhat carefully by Lubavin in 1870,† who described some of its properties. He considered this casein dyspeptone as a mixture of two distinct bodies, separable from each other by the action of sodium carbonate. Substance *A*, that portion of the dyspeptone soluble in sodium carbonate, was described by Lubavin as a body containing 4·6 per cent. of phosphorus, 13·3 per cent. of nitrogen, 48·5 per cent. of carbon and no sulphur, corresponding to the formula $C_{21}H_{41}N_8PO_{11}$. Substance *B*, insoluble in sodium carbonate, was slowly soluble in sodium hydroxide, contained sulphur, but only a small trace of phosphorus and evidently contained more or less

* Chittenden and Painter. Studies from Laboratory of Physiological Chemistry Yale University, vol. ii., p. 156.

† Hoppe-Seyler. Med. Chem. Untersuchungen, p. 463.

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admixture of fat. Judging from the description, substance *A* must have been an acid compound of the body studied, mixed with more or less undigested casein; while substance *B* was doubtless a mixture of fat and the body *A*. However this may be, our results show conclusively that no body having the formula ascribed by Lubavin to his substance *A*, can be separated from the undigestible residue of casein in pepsin-hydrochloric acid.

In all of the experiments to be described, the casein employed was freshly prepared from skim milk by precipitating the greatly diluted fluid with dilute acetic acid, washing thoroughly with water, redissolving the precipitate in water containing a trace of ammonia and reprecipitating, repeating this operation three or four times.

In subjecting casein to the action of artificial gastric juice the conditions were varied more or less in the individual experiments, so that if the so-called dyspeptone be a mixture of two or more substances, the varying conditions under which the digestions were made might so change the nature of the mixture, that on analysis, it would become apparent.

Digestion A.

The casein from five gallons of milk was placed in four litres of 0·4 per cent. hydrochloric acid and warmed to 40° C. To this was added 200 c. c. of a dialyzed pepsin solution, prepared from a glycerin extract of the ferment, and the mixture kept at 40–45° C. for forty-eight hours. At the end of this time there was still a comparatively large mass of gelatinous matter undissolved, composed in part, no doubt, of swollen casein. The entire mixture was then diluted considerably with water and treated with dilute alkali to near neutralization, leaving the fluid, however, distinctly acid. The undigested matter was then filtered off and washed thoroughly with water. This partial neutralization of the digestive mixture was found necessary, owing to the extreme slowness with which the acid fluid filtered. The undigested matter was again warmed at 40° C. for forty-eight hours, with four litres of a much more vigorous pepsin mixture containing 0·4 per cent. hydrochloric acid. The residue still undissolved was filtered off and washed with water. The acid filtrate gave no precipitate whatever on neutralization. A third time the undigested matter was warmed at 40° C. for sixty hours, with four litres of a still stronger artificial gastric juice. The quantity of insoluble matter did not appear to be diminished at all by this third treatment with pepsin and acid. The substance was thereupon filtered from the acid

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fluid, and washed with water until the washings gave no reaction for chlorides. It was then treated in the cold with one litre of one per cent. sodium carbonate, in which it appeared to dissolve completely. On filtration there appeared a small whitish residue, which on treatment with ether, dissolved in great part, thus showing its fatty nature. There remained, however, a very small residue of a heavy, brownish substance too small in quantity to be considered other than as an impurity. On adding dilute hydrochloric acid to the alkaline fluid, no precipitate was obtained until the fluid was made distinctly acid, when the dyspeptone was thrown down as a heavy flocculent precipitate. In the filtrate, the biuret and Millon's test showed only a faint trace of an albuminous body. The precipitate of dyspeptone was washed with water until the washings gave no reaction with silver nitrate, after which it was dissolved in one per cent. sodium carbonate, the fluid made exactly neutral with dilute hydrochloric acid, thymolized, and then dialyzed in running water until all chloride was removed from the fluid.

The neutral fluid of dyspeptone so obtained, was concentrated to a thick syrup on the water-bath, and then while still warm, it was treated with 95 per cent. alcohol and a little absolute alcohol. A moderately heavy precipitate of dyspeptone resulted, but apparently not all of the substance was precipitated. On standing for forty-eight hours, the fluid was found in a thick, gelatinous condition. The coagulum was insoluble in 95 per cent. alcohol, but readily and completely soluble in water. It was therefore washed thoroughly with alcohol, allowed to stand under absolute alcohol for several days, then treated with cold ether, after which it was dried, powdered and placed in a fat extractor and extracted with boiling ether as long as any fatty matter was dissolved, a process which took several days. About nine grams of the pure, dry substance were obtained.

A portion was then dried at 110° C. until of constant weight, for analysis. Its composition is shown in the accompanying table.

The methods of analysis employed were the same as those previously described. Phosphorus was determined by fusing the substance in a silver crucible with potassium hydroxide and potassium nitrate, acidifying the mixture with nitric acid, evaporating to dryness, dissolving the residue in water acidified with nitric acid and precipitation of the phosphoric acid, first with molybdic solution, and lastly with magnesia mixture and final weighing of the phosphorus as magnesium pyrophosphate.

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Dyspeptone A.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	$Mg_2P_2O_7$ from the Ash. gram.	P of Ash. %	$Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram.	P %	P after deducting P of Ash. %
I	0·2389	0·1325	6·04	0·3872	44·20	---	---	------	---	----	---	----	---	----	---	----	---	-----
II	0·3058	0·1695	6·16	0·4949	44·13	---	---	------	---	----	---	----	---	----	---	----	---	-----
III	0.4003	------	---	------	----	44·9	19·4	754·4	13·05	----	---	----	---	----	---	----	---	-----
IV	0·4581	------	---	------	----	51·0	17·8	760·0	13·14	----	---	----	---	----	---	----	---	-----
V	0·3834	------	---	------	----	---	---	------	---	0·0523	13·64	----	---	----	---	----	---	-----
VI	0·4092	------	---	------	----	---	---	------	---	0·0561	13·70	----	---	----	---	----	---	-----
VII	0·5884	------	---	------	----	---	---	------	---	----	---	0·0253	0·59	----	---	----	---	-----
VIII	0·5227	------	---	------	----	---	---	------	---	----	---	0·0243	0·63	----	---	----	---	-----
IX	0·4092	------	---	------	----	---	---	------	---	----	---	----	---	0·0379	2·58	----	---	-----
X	0·5884	------	---	------	----	---	---	------	---	----	---	----	---	----	---	0·0537	2·54	0
XI	0.5227	------	---	------	----	---	---	------	---	----	---	----	---	----	---	0·0501	2·67	0·09

Percentage composition of the ash-free substance.

							Average.
C	51·18	51·12	----	----	----	----	*51·15*
H	7·13	7·24	----	----	----	----	*7·18*
N	----	----	15·12	15·20	----	----	*15·16*
S	----	----	----	----	0·69	0·73	*0·71*
P	----	----	----	----	----	----	*0*
O	----	----	----	----	----	----	*25·80*
							100·00

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It is to be seen from the table of analytical results that the total phosphorus is exactly equal to the phosphorus of the ash. The ash, as examination showed, was composed almost entirely of calcium phosphate with a trace of iron. There was no calcium sulphate. Taking the percentage of phosphorus at the highest figure, viz: 2·67, and calculating it to calcium phosphate $Ca_3(Po_4)_2$, it would be equal to 13·3 per cent. of calcium phosphate, or within 0·3 per cent. of the ash found. Hence, it would appear that the phosphorus present in the substance probably existed there wholly as calcium phosphate.

Digestion B.

A quantity of pure casein, equal in amount to that used in digestion *A*, was warmed at 40° C. with seven litres of 0·4 per cent. hydrochloric acid, to which a quantity of purified and vigorous pepsin solution was added. After being kept at 40° C. for fifty hours, two litres more of 0·4 per cent. hydrochloric acid, together with some pepsin solution, were added and the mixture warmed at 40° C. for two days more, after which it was diluted with water and the undigested residue allowed to settle out. The supernatant fluid was syphoned off, the residue washed by decantation and then again treated at 40° C. with five litres of an active pepsin-hydrochloric acid solution for four days. The residue still undigested was filtered off, washed with water, dissolved in one per cent. sodium carbonate solution and the alkaline fluid filtered from the small amount of undissolved matter. From this fluid, the dyspeptone was precipitated by hydrochloric acid, the acid compound washed thoroughly with water, after which it was warmed at 40° C. for forty-eight hours with 1200 c. c. of 0·2 per cent. hydrochloric acid, and 50 c. c. of a strong pepsin solution. The undigested residue, after being thoroughly washed, was dissolved in 800 c. c. of one per cent. sodium carbonate, the solution exactly neutralized with hydrochloric acid and dialyzed until chlorides were entirely removed. The clear aqueous solution was evaporated to a syrup and the dyspeptone precipitated with alcohol, after which it was treated exactly as preparation *A*. 9·5 grams of pure, dried substance were obtained.

For analysis, the dyspeptone was dried at 110° C. until of constant weight. The analytical results are shown in the accompanying table.

The ash which was larger than in the first preparation, contained no sulphate whatever, but was composed in great part of calcium phosphate.

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Dyspeptone B.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	$Mg_2P_2O_7$ from the Ash. gram.	P of Ash. %	$Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram.	P %	P after deducting P of Ash. %
I	0·4220	0·2385	6·28	0·6680	43·16	---	---	------	---	----	---	----	---	----	---	----	---	-----
II	0·2814	0·1582	6·25	0·4465	43·27	---	---	------	---	----	---	----	---	----	---	----	---	-----
III	0·4918	------	---	------	----	55·1	20·2	754·7	13·02	----	---	----	---	----	---	----	---	-----
IV	0·3221	------	---	------	----	35·4	20·1	758·9	12·84	----	---	----	---	----	---	----	---	-----
V	0·4145	------	---	------	----	47·0	19·4	758·1	13·26	----	---	----	---	----	---	----	---	-----
VI	0·5141	------	---	------	----	---	---	------	---	0·0791	15·38	----	---	----	---	----	---	-----
VII	0·4893	------	---	------	----	---	---	------	---	0·0756	15·45	----	---	----	---	----	---	-----
VIII	0·5304	------	---	------	----	---	---	------	---	----	---	0·0223	0·58	----	---	----	---	-----
IX	0·5040	------	---	------	----	---	---	------	---	----	---	0·0228	0·62	----	---	----	---	-----
X	0·4893	------	---	------	----	---	---	------	---	----	---	----	---	0·0484	2·76	----	---	-----
XI	0·6304	------	---	------	----	---	---	------	---	----	---	----	---	----	---	0·0633	2·80	0·04
XII	0·6056	------	---	------	----	---	---	------	---	----	---	----	---	----	---	0·0586	2·72	0

Percentage composition of the ash-free substance.

								Average.
C	51·02	51·13	----	----	----	----	----	*51·07*
H	7·42	7·40	----	----	----	----	----	*7·41*
N	----	----	15·40	15·23	15·62	----	----	*15·41*
S	----	----	----	----	----	0·69	0·73	*0·71*
P	----	----	----	----	----	----	----	*0*
O	----	----	----	----	----	----	----	*25·40*
								100·00

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Digestions C and D.

These digestions were conducted in much the same manner as the preceding. In *C*, the casein was first subjected to the action of four litres of vigorous, but purified, artificial gastric juice containing 0·4 per cent. acid, for four consecutive days. The undigested residue was then filtered off, washed, and again treated with a vigorous pepsin-acid mixture for three days longer, in both cases at 40° C. The residue still undigested was washed thoroughly with water and then dissolved in one per cent. sodium carbonate, after which it was treated exactly as preparation A.

In *D*, the casein was warmed for three days with six litres of a similar pepsin-acid mixture, when the undissolved residue, after being filtered and thoroughly washed, was dissolved in diluted sodium carbonate and reprecipitated by dilute hydrochloric acid. Then, as in *B*, the washed precipitate was redigested with a vigorous gastric juice for several days and the residue again dissolved, after thorough washing, in one per cent. sodium carbonate and treated exactly as the preceding preparation.

For analysis, both products were freed entirely from fat, and ultimately dried at 110° C., until of constant weight. Their composition is shown in the accompanying tables.

Three other distinct preparations of dyspeptone were made in manner similar to the preceding, except that in all, larger quantities of pepsin-hydrochloric acid were employed and the mixtures warmed for a longer time at 40° C. Thus in digestion *E*, 1562 grams of moist casein were warmed with 9·5 litres of 0·4 per cent. hydrochloric acid and pepsin for two days, and the undigested residue again treated at 40° C. with 3 litres of a like pepsin-acid for seven days, and finally treated a third time with pepsin and acid for four days, before solution in sodium carbonate, etc. Likewise in digestion *G*, 2000 grams of moist casein were warmed at 40° C. with 11 litres of 0·4 per cent. acid and pepsin for 21 days and the residue warmed again at 40° C. for several days, with a fresh pepsin-acid mixture. Ultimately, all of the three products were treated as previously described, and finally dried at 110° C. prior to analysis. The analytical results are shown in the accompanying tables.

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DYSPEPTONE C.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T.°C.	N found. Pressure m.m.	N %	Ash found. gram.	Ash %	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	$Mg_2P_2O_7$ from the ash. gram.	P of ash %	$Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram.	P %	P after deducting P of ash. %
I	0·3603	0·2068	6·38	0·5946	45·03	---	---	----	---	----	---	----	---	---	---	----	---	-----
II	0·2349	0·1341	6·34	0·3857	44·80	---	---	----	---	----	---	----	---	---	---	----	---	-----
III	0·6983	----	----	----	----	78·8	18·4	759·5	13·28	----	---	----	---	----	---	----	---	-----
IV	0·4780	----	----	----	----	54·9	18·8	756·8	13·44	----	---	----	---	----	---	----	---	-----
V	0·5470	----	----	----	----	---	---	----	---	0·0680	12·43	----	---	----	---	----	---	-----
VI	0·5655	----	----	----	----	---	---	----	---	0·0703	12·43	----	---	----	---	----	---	-----
VII	0·5733	----	----	----	----	---	---	----	---	----	---	0·0245	0·58	----	---	----	---	-----
VIII	0·5908	----	----	----	----	---	---	----	---	----	---	0·0262	0·60	----	---	----	---	-----
IX	0·5655	----	----	----	----	---	---	----	---	----	---	----	---	0·0469	2·31	----	---	-----
X	0·5733	----	----	----	----	---	---	----	---	----	---	----	---	----	---	0·0508	2·47	0·16
XI	0·5908	----	----	----	----	---	---	----	---	----	---	----	---	----	---	0·0524	2·47	0·16

Percentage composition of the ash-free substance.

							Average.
C	51·40	51·18	----	----	----	----	*51·29*
H	7·28	7·24	----	----	----	----	*7·26*
N	----	----	15·16	15·31	----	----	*15·23*
S	----	----	----	----	0·67	0·69	*0·68*
P	----	----	----	----	----	----	*0*
O	----	----	----	----	----	----	*25·54*
							100·00

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Dyspeptone D.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T.°C.	N found. Pressure m.m.	N %	Ash found. gram.	Ash %	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	$Mg_2P_2O_7$ from the ash. gram.	P of ash %	$Mg_2P_2O_7$ after fusion with $KOH + KNO_3$. gram.	P %	P after deducting P of ash. %
I	0·2070	0·1200	6·44	0·3390	44·66	---	---	----	---	----	---	----	- -	----	---	----	---	-----
II	0·2158	0·1236	6·38	0·3510	44·37	---	---	----	---	----	---	----	---	----	---	----	---	-----
III	0·4238	----	----	----	----	48·0	18·4	757·8	13·29	----	---	----	---	----	---	----	---	-----
IV	0·2711	----	----	----	----	30·9	20·1	757·6	13·29	----	---	----	---	----	---	----	---	-----
V	0·2070	----	-- -	-- -	----	---	---	----	---	0·0294	14·20	---	---	----	---	----	---	-----
VI	0·2158	----	----	----	----	---	---	----	---	0·0306	14·18	----	---	----	---	----	---	-----
VII	0·6436	----	----	----	----	---	---	----	---	----	---	0·0302	0·64	----	---	----	---	-----
VIII	0·5613	----	----	----	----	---	---	----	---	----	---	0·0279	0·68	----	---	----	---	-----
IX	0·4228	----	----	----	----	---	---	----	---	----	---	----	---	0·0386	2·55	----	---	-----
X	0·6436	----	----	----	----	---	---	----	---	----	---	----	---	----	---	0·0554	2·40	0
XI	0·5613	----	----	----	----	---	---	----	---	----	---	----	---	----	---	0·0508	2·52	0

Percentage composition of the ash-free substance.

							Average.
C	51·97	51·68	----	----	----	----	*51·82*
H	7·47	7·41	----	----	----	----	*7·44*
N	----	----	15·48	15·48	----	----	*15·48*
S	----	----	----	----	0·76	0·80	*0·78*
P	----	----	----	----	----	----	*0*
O	----	----	----	----	----	----	*24·48*
							100·00

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Dyspeptone E.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure. mm.	N %	Ash found. gram.	Ash %
I	0·7272	0·3758	5·74	1·1528	43·28	----	----	----	----	----	----
II	0·3746	0·1927	5·71	0·5967	43·17	----	----	----	----	----	----
III	0·3177	----	----	----	----	34·06	13·0	759·6	12·84	----	----
IV	0·5570	----	----	----	----	58·33	12·2	760·3	12·61	----	----
V	0·3319	----	----	----	----	----	----	----	----	0·0496	14·94
VI	0·3330	----	----	----	----	----	----	----	----	0·0499	14·98

Percentage composition of ash-free substance.

					Average.
C	50·84	50·75	----	----	*50·80*
H	6·75	6·72	----	----	*6·73*
N	----	----	15·10	15·15	*15·12*

Dyspeptone F.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %
I	0·5375	0·2837	5·86	0·8772	44·50	----	----	----	----	----	----
II	0·5686	0·3039	5·93	0·9317	44·68	----	----	----	----	----	----
III	0·4649	----	----	----	----	50·3	12·4	764·0	13·11	----	----
IV	0·4100	----	----	----	----	44·7	13·0	765·5	13·16	----	----
V	0·3938	----	----	----	----	----	----	----	----	0·0536	13·61
VI	0·4347	----	----	----	----	----	----	----	----	0·0590	13·59

Percentage composition of ash-free substance.

					Average.
C	51·50	51·70	----	----	*51·60*
H	6·78	6·87	----	----	*6·83*
N	----	----	15·17	15·21	*15·19*

Dyspeptone G.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %
I	0·7545	0·3915	5·76	1·2148	43·90	----	----	----	----	----	----
II	0·4094	0·2137	5·79	0·6604	43·98	----	----	----	----	----	----
III	0·6268	----	----	----	----	70·6	13·8	760·5	13·56	----	----
IV	0·7803	----	----	----	----	88·8	14·0	760·3	13·61	----	----
V	0·4356	----	----	----	----	----	----	----	----	0·0552	12·66
VI	0·5654	----	----	----	----	----	----	----	----	0·0722	12·76

Precentage composition of ash-free substance.

					Average.
C	50·35	50·44	----	----	*50·39*
H	6·61	6·65	----	----	*6·63*
N	----	----	15·55	15·61	*15·58*

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TABLE SHOWING THE AVERAGE COMPOSITION OF THE SEVERAL DYSPEPTONES.

	A.	B.	C.	D.	E.	F.	G.	Casein.*
C	51·15	51·07	51·29	51·82	50·80	51·60	50·39	53·30
H	7·18	7·41	7·26	7·44	6·73	6·83	6·63	7·07
N	15·16	15·41	15·23	15·48	15·12	15·19	15·58	15·91
S	0·71	0·71	0·68	0·78	....	....	....	0·82
P	0	0	0	0	....	....	....	0·87
O	25·80	25·40	25·54	24·48	....	....	....	22·03
Ash	13·67	15·41	12·43	14·19	14·96	13·60	12·71	0·98

It is evident from the more complete analyses of the first four products, that the dyspeptone as prepared by us contains essentially the same percentage of sulphur as the original casein; further, that instead of being a phosphorized compound, it apparently contains no phosphorus whatever, other than that combined with calcium. Very noticeable, is the large percentage of ash in all of the preparations. This we were not able to materially reduce by any process of purification, and as the ash of the original casein, like that of the dyspeptone, was composed almost wholly of calcium phosphate, it would appear as if all of the phosphate from the mother substance had attached itself to the dyspeptone.

In their content of carbon, all of the seven preparations show a very close agreement, while they differ from casein by containing two per cent. less carbon. The nitrogen of the dyspeptone is, likewise, a little less than that of casein, while the individual preparations show throughout a very close agreement in their content of this element. In composition, therefore, all of the seven preparations, although representing considerable variation in the method of production, show a sufficiently close agreement to indicate their identity. Compared with casein, the lower percentage of carbon would point to their production by hydration, and it would appear from the analytical data that the so-called casein dyspeptone, formed by gastric digestion, is a mixture of calcium phosphate with a hydration product of casein,

* Chittenden and Painter. Studies from Laboratory, vol. ii. p. 173.

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the hydrochloric acid compound of which is insoluble in water and dilute acid. The dyspeptone itself is quite readily soluble in cold water, the solution remaining unchanged on boiling.

Following are some of the reactions of dyspeptone.

Addition of acetic acid to an aqueous solution of the substance produces a heavy white precipitate insoluble in moderate excess, but partially soluble in a large excess of the acid. On heating the strongly acid fluid, the precipitate dissolves completely and the fluid remains clear on cooling. Addition of potassium ferrocyanide to the clear acid fluid gives only a slight turbidity.

Dilute hydrochloric and sulphuric acid both give a heavy white precipitate, insoluble in slight excess of acid, but entirely soluble in a large excess on application of heat. Even 0·2 per cent. hydrochloric acid precipitates the dyspeptone completely.

Dilute nitric acid, likewise, precipitates the dyspeptone, but the precipitate is far more soluble in excess of the acid. On warming the acid solution, it quickly turns yellow, and with ammonia gives the orange yellow color of the xanthoprotein reaction.

Cupric sulphate and potassium hydroxide give the violet color of the biuret reaction.

Cupric sulphate and ferric chloride both give heavy precipitates, insoluble in excess.

Potassium hydroxide and lead acetate give, on boiling, a distinct reaction for sulphur.

Mercuric chloride, added in small quantity to a cold aqueous solution of the dyspeptone, gives no precipitate, but when added in excess and the mixture is heated, a heavy white precipitate is formed, insoluble on cooling.

The dyspeptone is precipitated by saturation of its aqueous solution with ammonium sulphate, but not by sodium chloride, even on heating. Addition of acetic acid, however, to the salt-saturated fluid gives the usual precipitate of dyspeptone.

Casein antialbumid.

On heating casein with sulphuric acid and water at 100° C., it is decomposed, as is well known, into soluble products and an insoluble antialbumid. We have found, however, that the antialbumid prepared in this manner is quite different in composition from the dyspeptone formed in gastric digestion. In one experiment, where about two kilograms of pure, moist casein were heated with two litres of water and 100 grams of concentrated sulphuric acid for seven hours

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at 100° C., and the residue so obtained treated again in a like manner with the same strength of acid, a comparatively large amount of casein antialbumid was obtained, which unlike the dyspeptone from a gastric digestion, was only slowly soluble in dilute sodium carbonate. Freed from any adhering soluble products by treatment with several litres of a vigorous gastric juice for two days at 40° C., it still dissolved slowly in dilute sodium carbonate. By long contact with a one per cent. solution of the alkaline carbonate it finally dissolved, leaving but a small residue. From this solution, the antialbumid was reprecipitated by hydrochloric acid, and after thorough washing with water, it was again dissolved in sodium carbonate, the fluid made exactly neutral and then dialyzed until all chloride was removed. After concentration of the fluid and precipitation with alcohol, etc., the antialbumid was dried and analyzed. It contained 1·8 per cent. of ash. The ash-free substance contained 54·4 per cent. of carbon, 6·8 per cent. of hydrogen, and 14·8 per cent. of nitrogen; showing thus a much higher percentage of carbon, and a lower percentage of nitrogen than the dyspeptone formed by pepsin-hydrochloric acid.

Both casein antialbumid and dyspeptone are dissolved with more or less readiness by alkaline solution of trypsin and are converted by long warming at 40° C. into a peptone-like body, presumably antipeptone.

II. *Caseoses;*— from experiments by *Charles Norris, Jr., Ph.B., and C. A. Tuttle, Ph.B.*

In a previous study of the caseoses formed in pepsin digestion,* we were much impressed with the peculiar behavior of protocaseose towards acids. Unlike the proto bodies from other proteids previously studied, *aqueous* solutions of the substance gave heavy precipitates with dilute acids. Protocaseose, as then separated, was readily soluble in 0·4 per cent. hydrochloric acid, but addition of stronger acid invariably produced a decided precipitate, soluble, however, in a still larger excess of acid. This peculiarity rendered the protocaseose an object of some interest to us, and further study of the conditions favoring its formation in gastric digestion has shown us that, apparently, the nature of the body precipitated by saturation with salt, as well as the body precipitated by salt-saturated acetic acid is modified by the strength of the pepsin solution,

* Chittenden and Painter. Studies from Laboratory of Physiological Chemistry, vol. ii, p. 195.

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and the length of time the casein is subjected to the action of the ferment. In our earlier work with the proteoses, we deemed it essential in attempting a study of the primary products of proteolytic action to use as weak a ferment solution as possible, and to discontinue its action as soon as solution of the proteid was complete, in order that there might not be too great a loss through formation of peptone. Further study, however, especially by use of the ammonium sulphate reaction, has shown us that the formation of peptone is a far less rapid process than generally supposed. Indeed, in the majority of artificial digestions with pepsin-hydrochloric acid, as ordinarily conducted, the ammonium sulphate reaction will show the entire absence of peptone. True peptone appears to be formed only by the action of a very vigorous pepsin mixture and that long continued.

In all of our previous experiments, the casein was either subjected to the action of a very weak pepsin mixture or else, in the use of a stronger ferment, exposed to its action for a few hours only. We now find that by using a far more vigorous pepsin mixture and by continuing its action for several days instead of hours, there is still not a trace of peptone to be found in the filtrate from the ammonium sulphate precipitate of the caseoses, but that the caseoses themselves, particularly the proto and deuterocaseose, differ somewhat, both in composition and reactions from the products previously separated. The discovery of this fact has led us to a further study of the caseoses formed in pepsin digestion, by which we have been able in many ways to verify our former observations and at the same time extend our knowledge of these interesting primary cleavage products of casein. We have also extended our work by studying the caseoses formed through the action of trypsin, and dilute sulphuric acid.

A. Caseoses formed by pepsin-hydrochloric acid.

In all of these experiments the pepsin mixture was very powerful, and was especially prepared to insure freedom from both albumoses and peptone.* The casein was, likewise, thoroughly pure, having been freshly prepared from skim milk by precipitation with 0·2 per cent. hydrochloric acid, and reprecipitation three or four times after solution in ammoniacal water.

* Studies from the Laboratory of Physiological Chemistry, vol. ii, p. 133.

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Digestion A.

Nearly 2 kilos. of moist casein were warmed at 40° C. for a little more than two days, with 10 litres of 0·4 per cent. hydrochloric acid containing sufficient of the pepsin mixture to insure vigorous action. After partial neutralization of the acid, the clear fluid was filtered from the semi-gelatinous dyspeptone, made exactly neutral with sodium hydroxide and then evaporated until moderately concentrated. On filtering the concentrated fluid through paper, a small residue remained, somewhat gummy, insoluble in dilute acid, but readily soluble in dilute sodium carbonate, from which it was precipitated by either hydrochloric or acetic acid. The amount was too small for study, but it seemed to resemble in reactions casein dyspeptone.

The neutral fluid containing the caseoses gave no precipitate whatever on addition of 0·4 per cent. hydrochloric acid or even stronger acid, and in this respect differs from the earlier digestions in which the ferment action was continued for a short time only. With dilute acetic acid, however, a slight turbidity was produced, the amount of which was too small to admit of any study of its character.

The caseoses were precipitated collectively in the form of a heavy gummy precipitate, by saturation of the neutral fluid with ammonium sulphate. On boiling the filtrate from this ammonium sulphate precipitate, a small quantity of a second gummy precipitate was obtained. In the filtrate from this second precipitate, no trace of a peptone-like body could be discovered by any of the ordinary tests. Apparently only caseose bodies had been formed.

The first and main ammonium sulphate precipitate, after being washed by trituration with a saturated solution of ammonium sulphate, was dissolved in water and the perfectly neutral fluid saturated in the cold with sodium chloride. By this means a heavy gummy precipitate was formed, which after being washed with a saturated solution of sodium chloride was redissolved in water, and reprecipitated by saturation of the neutral fluid with salt. After three or four reprecipitations, the protocaseose was considered sufficiently pure.

In this digestion, there appeared to be present more heterocaseose and dyscaseose than in our former experiments, as was evidenced by the small insoluble residues remaining each time the precipitated protocaseose was redissolved in water. These residues of hetero- and

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dyscaseose were very small, but still sufficiently large to enable us to make out their general characters. Similarly, Dr. Thierfelder* found in the purification of his protocaseose, or "propeptone I" as he terms it, a small, insoluble residue each time he dissolved the sodium chloride precipitate in water. It would appear, therefore, that in a vigorous or long continued digestion there is a much greater probability of the hetero body being formed than when the ferment is allowed to act only a short time on the casein. This, however, is contrary to Neumeister's views regarding the order of formation of the proteoses.

In order to free the precipitated protocaseose from salt and any adhering heterocaseose, it was dissolved in water and dialyzed until all chloride was removed. The neutral solution was then concentrated to a syrup, and the proto body precipitated by alcohol. During the concentration of the fluid, a gummy-like mass separated, similar to the separation of protoelastose. This, however, dissolved more or less completely as the mixture cooled. The precipitated caseose, after being extracted several times with alcohol and with warm ether, was partially dried, ground to a fine powder, re-extracted with ether in a fat extractor and finally dried at 110° C. until of constant weight.

On analysis it gave the following results:

Protocaseose A.

I. 0·4947 gram substance gave 0·9794 gram CO_2=53·98 per cent. C.

II. 0·3802 gram substance gave 0·2397 gram H_2O=7·00 per cent. H.

III. 0·3862 gram substance gave 0·2467 gram H_2O=7·09 per cent. H and 0·7670 gram CO_2=54·15 per cent. C.

IV. 0·4953 gram substance gave 64·8 c. c. N at 20·8° C. and 760·3 mm pressure=15·72 per cent. N.

V. 0·3764 gram substance gave 50·1 c. c. N at 22·5° C. and 760·6 mm pressure=15·95 per cent. N.

VI. 0·4599 gram substance gave 0·0046 gram ash=1·00 per cent.

VII. 0·4103 gram substance gave 0·0041 gram ash=0·99 per cent.

Percentage composition of ash-free substance.

						Average.
C	54·53	54·69	....	....	....	54·61
H	....	7·11	7·07	....	....	7·11
N	....	....	....	15·88	16·11	15·99

* Zur Kenntniss der Caseinpeptone, Zeitschrift für physiologische Chemie, x, p. 577.

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This protocaseose is thus seen to have a higher percentage of carbon than the proto bodies previously* studied, although one product was then obtained with 53·93 per cent. C, 7·17 per cent. H, and 16·05 per cent. N.

In reactions, likewise, this protocaseose differs somewhat from the protocaseoses previously obtained, and as the characters of the present body have been verified by the reactions of several other products similarly produced, we are led to believe in their constancy. Moistened with water, the powdered protocaseose becomes immediately gummy and soon dissolves to a perfectly clear fluid, which on addition of considerable water becomes decidedly cloudy or turbid. Treated with a large amount of water at the outset, the protocaseose dissolves more slowly, giving a more or less turbid fluid. In dilute acid and in dilute sodium carbonate it dissolves to a perfectly clear fluid.

Towards heat, aqueous solutions of protocaseose act exactly like protoelastose. Even when warmed very gently, the solution becomes quickly turbid and if concentrated gives more or less of a flocculent precipitate. On cooling, the turbidity disappears, reappearing as the fluid is heated. Like protoelastose also, a solution of the caseose body on being rapidly concentrated deposits more or less of the substance as a gummy mass, which, however, will dissolve in cold water, or if the fluid is not too concentrated will dissolve in the mother liquid as it cools. Dilute acetic acid added to an aqueous solution of protocaseose gives no precipitate whatever, but potassium ferrocyanide added to the acid fluid gives a heavy precipitate.

Dilute hydrochloric acid produces no precipitate.

Dilute nitric acid added to an aqueous solution of the caseose gives a heavy white precipitate, which on gently warming, quickly dissolves while the fluid takes on a faint pink or rose color, which on further warming changes to a bright yellow or reddish yellow color. If the nitric acid solution is not warmed too long, the precipitate reappears as the mixture cools.

Solution of cupric sulphate gives a heavy, greenish white precipitate when added to an aqueous solution of protocaseose.

As already stated, the first precipitates of protocaseose obtained by saturating the neutralized digestive fluid with salt, were not entirely soluble in water; a small residue remained, apparently

* See Chittenden and Painter. Studies from Laboratory of Physiological Chemistry, Yale University, vol. ii, p. 197.

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wholly insoluble. This residue, which by analogy should consist of hetero and dyscaseose was somewhat soluble in salt solution and wholly soluble in dilute sodium carbonate and dilute acid, both acetic and hydrochloric, even dissolving in 0·2 per cent. hydrochloric acid. It was dissolved in sodium carbonate, the fluid neutralized without giving any precipitate, and dialyzed until the sodium chloride was entirely removed. The neutral and clear fluid was then concentrated to a syrup, precipitated by alcohol and the precipitate dried at 110° C. On treatment with cold water, it was now found soluble to a large extent, though a certain amount of gummy matter was still insoluble. The soluble portion showed all of the reactions of the proto body; the solution being rendered turbid by heat, clear again on cooling, and giving in the cold a heavy precipitate with nitric acid, soluble when heated. This behavior of the insoluble heterocaseose towards dilute sodium carbonate would seem to imply a conversion of this substance into protocaseose, or perhaps a reconversion of the coagulated heterocaseose dyscaseose, into heterocaseose proper.

Acetic acid precipitate.

On adding a little 30 per cent. acetic acid to the original salt-saturated filtrate from the first sodium chloride precipitate of protocaseose, etc., a heavy, flocculent precipitate settled out, which in amount far exceeded the protocaseose and which on standing, soon became gummy. Excess of acid was avoided, as the precipitate was somewhat soluble in a large amount of the reagent. The gummy mass, after being washed as thoroughly as possible with saturated salt solution, was treated with cold water, in which the greater portion of the substance dissolved, the solution made neutral, dialyzed, concentrated to a syrup, and the substance precipitated with alcohol. It was then thoroughly extracted with ether and finally dried at 110° C.

In reactions, it differed decidedly from protocaseose; in water it was quickly and completely soluble and the solution when heated gave no coagulum whatever, or at the most only the slightest approach to a turbidity.

Dilute nitric acid, in the cold, gave no precipitate; when heated, the acid fluid changed to a reddish yellow color, which quickly turned yellow.

With acetic acid, an aqueous solution of the substance remained perfectly clear. Potassium ferrocyanide, however, when added to the acid fluid gave a heavy precipitate.

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Saturation of the aqueous solution with salt gave a slight turbidity.

Cupric sulphate gave a heavy precipitate, soluble in excess.

On analysis, the substance gave the following results, which show a composition quite different from that of protocaseose.

Acetic Acid Precipitate A.

I. 0·4629 gram substance gave 0·2812 gram H_2O=6·74 per cent. H and 0·8608 gram CO_2=50·71 per cent. C.

II. 0·3277 gram substance gave 0·1977 gram H_2O=6·70 per cent. H and 0·6050 gram CO_2=50·34 per cent. C.

III. 0·4253 gram substance gave 53·1 c. c. N at 13·0° C. and 761·8 mm pressure=15·01 per cent. N.

IV. 0·4843 gram substance gave 60·8 c. c. N at 13·0° C. and 760·8 mm pressure=15·07 per cent. N.

V. 0·3649 gram substance gave 0·0108 gram ash=2·96 per cent.

VI. 0·3947 gram substance gave 0·0124 gram ash=3·14 per cent.

Percentage composition of ash-free substance.

					Average.
C	52·30	51·92	----	----	*52·10*
H	6·96	6·90	----	----	*6·93*
N	----	----	15·48	15·54	*15·51*

As previously stated, the above original precipitate produced by acetic acid was not entirely soluble in water. A small residue remained, which after being washed with water was dissolved in dilute sodium carbonate, and the solution neutralized with dilute hydrochloric acid, without yielding any neutralization precipitate. The solution was then dialyzed, concentrated to a syrup and the substance precipitated by alcohol. It was now found, to a great extent, soluble in water, the solution showing no turbidity by heat and giving no precipitate with nitric acid. Cupric sulphate gave a heavy precipitate, and dilute acetic acid added to the aqueous solution produced quite a heavy precipitate, not readily soluble in excess of the acid. The amount of substance was too small to admit of analysis, and the reactions are hardly sufficient to identify it. It is evidently not heterocaseose, for it is only the acetic acid compound that is insoluble in water, not the caseose substance itself. In many respects it appears like casein dyspeptone, and as this substance is precipitated by saturation of its aqueous solution with ammonium sulphate and not by sodium chloride, the presence of a trace of this body might not be impossible.

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Ammonium sulphate precipitate.

On adding ammonium sulphate in substance to the above salt-saturated acetic acid fluid, a slight gummy precipitate was obtained, readily and completely soluble in water, and which, after removal of the salts by dialysis and concentration of the fluid, was precipitated by alcohol. In reactions it did not differ materially from the body obtained by precipitation with acetic acid, except that with cupric sulphate only a slight precipitate was produced. With acetic acid and potassium ferrocyanide, on the other hand, a distinct precipitate was obtained, while with nitric acid in the cold no turbidity whatever was produced.

In composition, however, it differed decidedly from the preceding preparations, although as it contained considerable ash, nearly 10 per cent., the result perhaps can be considered only as an approximation to the truth.

Following is the percentage composition of the ash-free substance:

					Average.
C	48.33	48·13	----	----	*48·23*
H	6·88	7·01	----	----	*6·94*
N	----	----	15·63	15·75	*15·69*

This body, which by analogy should be nearly pure deutero-caseose, evidently approaches much nearer to our conception of a true casein peptone than any of the preceding preparations. Another body, however, has been obtained with a still lower content of carbon and with reactions still more closely approximating to true peptone.

In the first precipitation of the caseoses from this digestion by saturation of the original fluid with ammonium sulphate, it will be remembered that a small amount of a second gummy precipitate was obtained on heating the cold saturated ammonium sulphate filtrate. This gummy precipitate of a caseose body, after purification by dialysis and precipitation with alcohol, was found to consist of a substance extremely soluble in water, the solution giving no precipitate with acetic acid and potassium ferrocyanide, neither with nitric acid nor with cupric sulphate. After being dried at 110° C. it gave on analysis the following results:

I. 0·7280 gram substance gave 0·4239 gram H_2O=6·46 per cent. H and 1·2160 grams CO_2=45·54 per cent. C.

II. 0·5020 gram substance gave 0·2905 gram H_2O=6·40 per cent. H and 0·8442 gram CO_2=45·85 per cent. C.

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III. 0·3291 gram substance gave 42·1 c. c. N at 13·9° C. and 756 mm pressure=15·33 per cent. N.

IV. 0·6835 gram substance gave 87·0 c. c. N at 14·5° C. and 756 mm pressure=15·30 per cent. N.

V. 0·5370 gram substance gave 0·0230 gram ash=4·28 per cent.

VI. 0·3525 gram substance gave 0·0154 gram ash=4·36 per cent.

Percentage composition of ash-free substance.

					Average.
C	47·57	47·87	----	----	*47·72*
H	6·75	6·70	----	----	*6·73*
N	----	----	15·99	15·96	*15·97*

This substance, since it is precipitable by ammonium sulphate, cannot be considered a true peptone, yet in composition it closely approaches both the ampho- and antipeptone from fibrin,* which it also resembles somewhat in reactions, except in its behavior towards ammonium sulphate.

It is thus evident from the foregoing, that in this active and comparatively long continued digestion there is a much smaller amount of protocaseose present than was found in our former experiments. Indeed, deuterocaseose appears to predominate, while at the same time the protocaseose is modified both in composition and reactions, due in part without doubt, to adhering heterocaseose. Further, we are inclined to consider the presence of at least two forms of deuterocaseose. We have generally considered that a proto body is never completely precipitated by saturation of its aqueous solution with salt, and that consequently the precipitate produced by acid in the salt-saturated fluid must be a mixture of proto and deuteroproteose, and this we have usually found to be the case. In the present digestion, however, the acetic acid precipitate contained only a very small amount of protocaseose, for as previously stated this precipitate when purified gave only a slight turbidity on saturation of its aqueous solution with salt and no precipitate whatever with nitric acid; both of which reactions would indicate freedom from any large amount of protocaseose. This view is further substantiated by the great difference in the percentage of carbon of the two bodies. To be sure we have, with Neumeister, looked on the cupric sulphate reaction as a means of distinguishing between proto and deuteroalbumose, but it does not necessarily follow that the same reaction will hold good for all proteoses. The acetic acid precipitate does indeed give a strong

* Kühne and Chittenden, Studies, vol. ii, p. 40.

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reaction with cupric sulphate, but the striking differences in composition and reactions between the purified sodium chloride precipitate and the acetic acid precipitate (when freed from acid) point to a totally different nature, and we are inclined to consider the latter as a deutero body, probably contaminated with a little protocaseose, and for convenience we propose to call it *a* deuterocaseose.

It is unquestionably very difficult, if not almost impossible, to isolate the individual caseoses in a state of perfect purity. Whenever one is precipitated, it usually brings down with it more or less of any other caseose present and such admixtures are very hard to remove. It is, we think, owing to this fact that we have not been able to obtain *a* deuterocaseose sufficiently free from protocaseose as not to give any precipitate on saturation with sodium chloride.

That form of caseose in this digestion which was not precipitated by salt, or by salt and acetic acid, but which appeared on addition of ammonium sulphate in the cold, is probably a mixture of *a* deutero and and what we term *β* deuterocaseose, with possibly an intermediate body.

The name *β* deuterocaseose, we apply to that caseose not readily precipitable by saturation with ammonium sulphate in the cold, and which is generally found in a greater or less quantity in the filtrate from the precipitate produced by saturation with ammonium sulphate. It is precipitated fairly pure, as a sticky gum, by simply boiling the saturated ammonium sulphate filtrate and is especially characterized by its low content of carbon, and by its non-precipitation with acetic acid and potassium ferrocyanide, with nitric acid, and with cupric sulphate. It stands, unquestionably, nearer to peptone than *a* deuterocaseose and is doubtless formed from the latter by the continued action of the ferment.

Digestion B.

In this digestion, 2 kilos. of moist casein were warmed at 40° C. for eight days, with about 5 litres of 0·4 per cent. hydrochloric acid containing an active pepsin solution, after which the mixture was partially neutralized with sodium carbonate and filtered from the dyspeptone. The clear fluid was then made exactly neutral (no neutralization precipitate) and concentrated to a thin syrup. When cold, proto and heterocaseose were directly precipitated by saturation of the solution with salt. Protocaseose was purified by repeated precipitation with salt, etc., as described under *A*. In this process, the same insoluble residues of hetero and dyscaseose were met with

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as in the previous digestion. The purified protocaseose showed the same peculiar reactions as protocaseose *A.*, viz: with nitric acid a heavy white precipitate, soluble on warming; with heat alone, a distinct turbidity or coagulum, disappearing as the solution cooled; and giving with a large amount of water a more or less turbid fluid, from which on standing a little gummy matter separated.

Dried at 110° C. and analyzed, the following results were obtained.

Protocaseose B.

I. 0·3470 gram substance gave 0·2192 gram H_2O=7·02 per cent. H.

II. 0·2935 gram substance gave 0·1854 gram H_2O=7·01 per cent. H and 0·5779 gram CO_2=53·69 per cent. C.

III. 0·2952 gram substance gave 0·5853 gram CO_2=54·07 per cent. C.

IV. 0·2989 gram substance gave 38·6 c. c. N at 13·8° C. and 760·1 mm pressure=15·52 per cent. N.

V. 0·5564 gram substance gave 72·8 c. c. N at 13·1° C. and 760·1 mm pressure=15·69 per cent. N.

VI. 0·3594 gram substance gave 0·0047 gram ash=1·30 per cent.

VII. 0·3523 gram substance gave 0·0045 gram ash=1·27 per cent.

Percentage composition of ash-free substance.

						Average.
C	----	54·77	54·39	----	----	54·58
H	7·10	7·09	----	----	----	7·10
N	----	----	----	15·71	15·90	15·80

In composition, therefore, as in reactions, this body is apparently identical with protocaseose *A.*

In the original salt-saturated filtrate from protocaseose, acetic acid produced a heavy, gummy precipitate, which was dissolved in water and purified in the same manner as the corresponding body in digestion *A.* Like the latter, it was readily and completely soluble in water, the solution giving no turbidity whatever by heat, nor on the addition of either nitric or acetic acid. With cupric sulphate, a heavy precipitate was formed, as also with acetic acid and potassium ferrocyanide.

Dried at 110° C. it gave the following results on analysis:

Acetic acid precipitate B.

I. 0·3429 gram substance gave 0·2057 gram H_2O=6·66 per cent. H and 0·6315 gram CO_2=50·22 per cent. C.

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II. 0·4345 gram substance gave 0·2734 gram H_2O=6·72 per cent. H and 0·8358 gram CO_2=50·48 per cent. C.

III. 0·3316 gram substance gave 43·5 c. c. N at 13·7° C. and 756·3 mm pressure=15·70 per cent. N.

IV. 0·3258 gram substance gave 43·1 c. c. N at 13·2° C. and 759·1 mm pressure=15·86 per cent. N.

V. 0·3306 gram substance gave 0·0126 gram ash=3·81 per cent.

VI. 0·2262 gram substance gave 0·0085 gram ash=3·76 per cent.

Percentage composition of ash-free substance.

					Average.
C	52·17	52·43	----	----	*52·30*
H	6·92	6·99	----	----	*6·95*
N	----	----	16·32	16·49	*16·40*

This body appears to differ from *a* deuterocaseose obtained in digestion *A* by nearly 1 per cent. of nitrogen, but in all other respects is practically identical with it.

As in digestion A, the precipitate first obtained by the addition of acetic acid was not entirely soluble in water, a residue remained soluble in dilute sodium carbonate, and which comported itself exactly like the insoluble residue obtained in the preceding digestion, apparently being a trace of casein dyspeptone.

The original filtrate from the above acetic acid precipitate, on saturation with ammonium sulphate, gave an additional precipitate, hardly sufficient for analysis, but which when purified proved to be identical with the corresponding body from *A* and like it giving with cupric sulphate only a very slight precipitate.

It is thus seen that in a vigorous pepsin digestion of casein there are formed, in addition to dyspeptone and heterocaseose, at least three distinct caseoses all soluble and differing from each other both in composition and reactions. As compared with casein, protocaseose is somewhat peculiar in containing a higher percentage of carbon than the mother substance. All of the other products show a very much smaller content of carbon. The relative composition of the products is shown in the following table:

	Casein.	*Proto-caseose A.*	*Proto-caseose B.*	*a Deutero-caseose A.*	*a Deutero-caseose B.*	*β Deutero-caseose A.*
C	53·30	54·61	54·58	52·10	52·80	47·72
H	7·07	7·11	7·10	6·93	6·95	6·73
N	15·91	15·99	15·80	15·51	16·40	15·97

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Protocaseose agrees closely in its content of carbon with the "propeptone I" of Thierfelder.* This investigator has separated from a pepsin digestion of casein, by a process similar to our method of separating the proto body, a substance to which he gives the above name and which contained 54·63 per cent. of carbon and 7·45 per cent. of hydrogen. In reactions it was similar to protocaseose, except that aqueous solutions of the substance remained perfectly clear on warming. Nitrogen was not determined. Thierfelder also separated from the filtrate from his "propeptone I," a second body, by addition of hydrochloric acid, to which he gives the name "propeptone II." This substance, which corresponds to our *a* deuterocaseose, he found to contain 49·8 per cent. carbon, 7·18 per cent. hydrogen and 14·23 per cent. nitrogen. Judging from the method of separation, however, the body analyzed must have been an acid compound of the caseose and not the caseose body itself. In reactions, so far as they are given, the substance was not different from *a* deuterocaseose.

b. Caseoses from Weyl's casein peptone.

This commercial product, sent to us from Germany, we have examined according to the foregoing methods and have found it, as might be expected, composed almost entirely of caseoses. It was completely soluble in water and gave with acetic acid a slight turbidity, somewhat increased by addition of potassium ferrocyanide. By saturation of its aqueous solution with sodium chloride, only a comparatively small precipitate was obtained, greatly increased, however, by addition of acetic acid.

200 grams of the powder were dissolved in water and the caseoses precipitated collectively by saturation of the fluid with ammonium sulphate, in the form of a heavy gummy precipitate. On heating the filtrate from this precipitate of caseoses until a crust of ammonium sulphate formed on the surface of the hot fluid, a second gummy precipitate gradually separated, which after purification by dialysis, etc., was finally precipitated by alcohol, and a portion dried at 110° C. for analysis.

This substance, representing a form of caseose not readily precipitable by ammonium sulphate and thus indicating its close approach to true peptone, is apparently identical with the *β* deuterocaseose similarly obtained in our own digestion, but present here in much

* Zeitschrift für physiologische Chemie, Band x, p. 585.

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larger quantity. In water it was readily and completely soluble, the solution giving no turbidity whatever by heat, nor with dilute nitric acid. With cupric sulphate only a very slight turbidity was produced, and with acetic acid and potassium ferrocyanide a correspondingly slight turbidity. Its aqueous solution on being saturated with salt and then made slightly acid with acetic acid showed a small flocculent precipitate, doubtless representing the substance which gave the slight turbidity with cupric sulphate and potassium ferrocyanide, viz: *a* deuterocaseose. On analysis the following results were obtained:

β deuterocaseose from Weyl's casein peptone.

I. 0·3641 gram substance gave 0·2121 gram H_2O=1·47 per cent. H and 0·6087 gram CO_2=45·58 per cent. C.

II. 0·4600 gram substance gave 0·2708 gram H_2O=6·54 per cent. H and 0·7652 gram CO_2=45·36 per cent. C.

III. 0·3298 gram substance gave 42·2 c. c. N at 13·9° C. and 755·4 mm pressure =15·31 per cent. N.

IV. 0·3418 gram substance gave 43·5 c. c. N at 14·4° C. and 756·1 mm pressure =15·31 per cent. N.

V. 0·3514 gram substance gave 0·0153 gram ash =4·35 per cent.

VI. 0·3599 gram substance gave 0·0155 gram ash =4·30 per cent.

Percentage composition of ash-free substance.

					Average.
C	47·61	47·40	----	----	*47·50*
H	6·76	6·83	----	----	*6·79*
N	----	----	16·00	15·84	*15·92*

In composition, therefore, as well as in reactions, this body resembles the *β* deutero described under *A*, and like it is especially characterized by its exceedingly low percentage of carbon.

From the first ammonium sulphate precipitate of caseoses, protocaseose was separated by saturation of the aqueous solution of the above precipitate with salt. As in similar precipitates from the preceding digestions, there was considerable heterocaseose present, showing itself as an insoluble residue when the sodium chloride precipitate was dissolved in water for reprecipitation. Purified after the methods previously described, the protocaseose showed the usual reactions characteristic of this body, its aqueous solution growing turbid when heated, giving a precipitate with nitric acid, etc.

Dried at 110° C. it gave on analysis the following results:

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Protocaseose, from Weyl's casein peptone.

I. 0·4291 gram substance gave 0·2597 gram H_2O=6·72 per cent. H and 0·8140 gram CO_2=51·70 per cent. C.

II. 0·3647 gram substance gave 0·2155 gram H_2O=6·56 per cent. H and 0·6935 gram CO_2=51·85 per cent. C.

III. 0·4115 gram substance gave 52·5 c. c. N at 13·6° C. and 760·8 mm pressure=15·11 per cent. N.

IV. 0·7730 gram substance gave 96·9 c. c. N at 13·8° C. and 761·2 mm pressure=15·10 per cent N.

V. 0·2901 gram substance gave 0·0121 gram ash=4·16 per cent.

VI. 0·2959 gram substance gave 0·0120 gram ash=4·05 per cent.

Percentage composition of ash-free substance.

					Average.
C	53·98	54·08	----	----	*54·01*
H	7·01	6·84	----	----	*6·92*
N	----	----	15·75	15·70	*15·72*

Addition of salt-saturated acetic acid to the filtrate from protocaseose, gave a moderately heavy, gummy precipitate which, after purification and removal of the acid, was completely soluble in water, the solution showing no turbidity by heat, but giving a slight turbidity with dilute nitric acid. It gave all of the reactions mentioned as characteristic of this precipitate. It was not analyzed.

The original filtrate from the acetic acid precipitate gave on saturation with ammonium sulphate, a small gummy precipitate which after purification was found wholly soluble in water, the solution giving no turbidity by heat and no precipitate with nitric or acetic acid. With acetic acid and potassium ferrocyanide, however, a distinct turbidity was produced and with cupric sulphate a heavy precipitate. The substance was not analyzed.

It is thus evident that Weyl's so-called casein peptone, however it may be prepared, contains essentially the same kind of caseoses found in our own digestions with pepsin-hydrochloric acid. In addition, a small amount of a substance was found not precipitable by saturation with ammonium sulphate and which gave no precipitate with nitric or acetic acid, nor with acetic acid and potassium ferrocyanide, and with cupric sulphate only a slight turbidity. This body, which doubtless was amphopeptone mixed with a little caseose, was obtained in too small quantity for analysis.

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c. Caseoses formed by dilute sulphuric acid at 100° *C.*

Two kilos. of pure moist casein were heated in a flask with 2 litres of water and 100 grams of pure sulphuric acid at 100° C. for seven hours. The residue of casein and antialbumid was again warmed with a like amount of fresh acid and water for six hours. The acid fluids were united, neutralized with sodium carbonate without giving any noticeable precipitate, and then evaporated until moderately concentrated. On cooling, considerable tyrosin and leucin crystallized from the fluid. With cupric sulphate, the solution gave a heavy precipitate which dissolved in sodium hydroxide with a reddish color. Sodium chloride and ammonium sulphate both produced heavy precipitates when added to saturation.

The caseoses were separated from the fluid by saturation in the cold with ammonium sulphate, and from this precipitate, protocaseose was separated by solution in water and precipitation with sodium chloride. The sodium chloride precipitate was dissolved in water, the substance reprecipitated by saturation with salt, and again dissolved in water to which a trace of sodium carbonate was added to make the mixture quite neutral. On dialysis, a small amount of gummy heterocaseose separated, mixed with a little tyrosin. The clear fluid, now free from salts, was concentrated on a water-bath. As the evaporation advanced, a brown gummy mass settled out, which was, however, readily soluble in cold water, for as the concentrated fluid cooled at night the gummy matter entirely disappeared, reappearing as the fluid was again heated. The final concentrated fluid was treated with alcohol, the gummy precipitate boiled with alcohol repeatedly to free it from any adhering tyrosin, and finally extracted with ether and dried at 110° C.

Analyzed, it gave the following results :

Protocaseose, formed by sulphuric acid.

I. 0·4405 gram substance gave 0.2718 gram H_2O=6·85 per cent. H and 0·8756 gram CO_2=54·20 per cent. C.

II. 0·3783 gram substance gave 0·2341 gram H_2O=6·87 per cent. H and 0.7604 gram CO_2=54·81 per cent. C.

III. 0·3786 gram substance gave 48·3 c. c. N at 17·5° C. and 753·4 mm pressure=14·93 per cent. N.

IV. 0·3780 gram substance gave 47·8 c. c. N at 17·0° C. and 754·0 mm pressure=14·84 per cent. N.

V. 0·3701 gram substance gave 0·0113 gram ash=3·05 per cent.

VI. 0·5112 gram substance gave 0·0154 gram ash=3·02 per cent.

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Percentage composition of ash-free substance.

					Average.
C	55·90	56·50	....	....	*56·20*
H	7·07	7·09	....	....	*7·08*
N	....	....	15·40	15·31	*15·36*

After being dried at 110° C. the substance dissolved with difficulty in water, leaving a large residue, but was readily and completely soluble in 0·2 per cent. hydrochloric acid and in 0·2 per cent. sodium carbonate. From the alkaline solution it was reprecipitated by hydrochloric acid, and not readily dissolved by an excess of the acid. Aqueous solution of the substance was rendered turbid by heat, the turbidity disappearing as the solution cooled. With dilute nitric acid, a white precipitate was formed, readily soluble on warming and reappearing as the solution cooled. Acetic acid and potassium ferrocyanide gave a heavy precipitate.

Thus in many respects this body resembles protocaseose formed by pepsin-hydrochloric acid, but is apparently characterized by a somewhat higher percentage of carbon.

The filtrate from the original sodium chloride precipitate, treated with salt-saturated 30 per cent. acetic acid, gave a flocculent, changing to gummy precipitate which was readily and completely soluble in water. The solution made exactly neutral was dialyzed without showing any evidence of a hetero-like body, finally concentrated and precipitated by alcohol. After being dried at 110° C., the substance was only partially soluble in water. The aqueous solution was rendered turbid by heat, clear again on cooling, and gave with nitric acid a heavy white precipitate as also with acetic acid and potassium ferrocyanide.

Analyzed, it gave the following results:

Acetic acid precipitate of caseose (a deuterocaseose?) formed by dilute sulphuric acid.

I. 0·4056 gram substance gave 0·2383 gram H_2O=6·52 per cent. H and 0·7733 gram CO_2=51·99 per cent. C.

II. 0·4697 gram substance gave 0·2740 gram H_2O=6·48 per cent. H and 0·8910 gram CO_2=51·73 per cent. C.

III. 0·5379 gram substance gave 66·5 c. c. N at 14·9° C. and 752·1 mm pressure=14·58 per cent. N.

IV. 0·4150 gram substance gave 0·0210 gram ash=5·06 per cent.

V. 0·5136 gram substance gave 0·0247 gram ash=4·80 per cent.

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Percentage composition of ash-free substance.

				Average.
C	54·68	54·42	....	*54·55*
H	6·86	6·81	....	*6·84*
N	....	....	15·83	*15·33*

This caseose, instead of being a pure deutero body, appears to be a mixture of proto and *a* deutero as indicated by its behavior towards heat and nitric acid; indeed, the behavior of this acetic acid precipitate resembles the acetic acid precipitate of caseose obtained in our previous work, where the two bodies were plainly precipitated together. In composition, while it agrees closely with protocaseose formed by pepsin-hydrochloric acid, it has a higher content of carbon than the corresponding *a* deutero, but bears about the same relation to the sulphuric acid protocaseose as the *a* deutero formed by pepsin to its corresponding protocaseose.

On boiling the original ammonium sulphate-saturated filtrate, a second gummy precipitate gradually separated from the hot fluid. This caseose, after purification by the usual methods and drying at 110° C., was entirely soluble in hot and cold water, and was especially characterized by yielding with acetic acid a heavy white precipitate, soluble in excess of the acid. With nitric acid it also gave a white precipitate, soluble in excess of acid. Cupric sulphate likewise gave a heavy precipitate. The cold water solution was not rendered turbid by heat.

Analyzed it gave the following results:

β deuterocaseose, formed by dilute sulphuric acid.

I. 0·3952 gram substance gave 0·2308 gram H_2O=6·49 per cent. H and 0·7282 gram CO_2=50·25 per cent. C.

II. 0·3518 gram substance gave 0·2064 gram H_2O=6·51 per cent. H and 0·6440 gram CO_2=49·92 per cent. C.

III. 0·4727 gram substance gave 59·2 c. c. N at 15·0° C. and 754·9 mm pressure = 14·81 per cent. N.

IV. 0·4279 gram substance gave 0·0233 gram ash = 5·44 per cent.

V. 0·5184 gram substance gave 0·0219 gram ash = 5·38 per cent.

Percentage composition of ash-free substance.

				Average.
C	53·10	52·77	....	*52·93*
H	6·86	6·89	....	*6·87*
N	....	....	15.66	*15·66*

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Thus, this body, which corresponds to the β deuterocaseose formed by pepsin-acid, has a relatively higher content of carbon and also varies in certain of its reactions, which resemble rather those of α deutero, and even the protocaseose of a pepsin digestion, than those of a genuine β deutero.

Altogether, the three caseoses separated from the sulphuric acid solution of casein, while showing a certain general relationship to the caseoses formed by pepsin-hydrochloric acid, are sufficiently different in their individual reactions to suggest at least some difference in their nature.

d. Caseoses formed by the action of trypsin.

In subjecting casein to the action of trypsin, care was taken that the ferment solution should be as free as possible from all products of the self-digestion of the pancreatic tissue. Dried pancreas from the ox, prepared according to Kühne's method, was warmed with 0·1 per cent. salicylic acid at 40° C. for 24 hours, after which the acid extract was neutralized and made alkaline with sodium carbonate to the extent 0·3 per cent. The alkaline solution, well thymolized, was warmed at 40° C. for several days in order to convert the albuminous matters present into easily diffusible products, after which it was dialyzed in running water for some time, and the solution ultimately evaporated to dryness at 40° C. This residue, being extracted with a small volume of water, gives a fairly pure solution of trypsin, free from objectionable impurities.

In the formation of the caseoses, 2200 grams of pure, moist casein were soaked in 3 litres of 1 per cent. sodium carbonate for several days, and the mixture well thymolized to prevent putrefaction. The trypsin solution was then added, together with some water, and the whole warmed at 40° C. At first, the mixture was quite limpid, the casein being dissolved in the alkaline fluid, but after two or three days, gelatinous lumps began to appear on the bottom of the dish and finally a soft coagulum appeared on the surface and all through the mixture, resembling the separation of antialbumid. This coagulum gradually disappeared and at the end of five days the digestion was stopped, and the alkaline fluid filtered from the undigested residue. The latter was thoroughly washed with thymolized water and the washings added to the filtrate. This residue of undigested matter was found to be insoluble in 0·5 per cent. sodium carbonate and also in 0·2 per cent. hydrochloric acid, but on being warmed with pepsin-hydrochloric acid it was in time almost completely dissolved.

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For separation of the caseoses the digestive fluid was neutralized with dilute hydrochloric acid, giving only a slight neutralization precipitate, and the neutral fluid concentrated. When moderately concentrated, the solution was placed in a cool place for several days to allow as much of the leucin and tyrosin to crystallize as possible. The caseoses were then separated from the filtrate, in the form of a gummy precipitate, by saturation of the fluid with ammonium sulphate. On boiling the filtrate from the first precipitate, and adding still more ammonium sulphate, a second gummy precipitate gradually settled out of the hot saturated fluid. This precipitate was separated from the fluid, the latter being saved for the detection of any peptone formed, washed with hot saturated ammonium sulphate solution, then dissolved in water and dialyzed until all traces of sulphate were removed. In the dialysis, no signs of any gummy heterocaseose or other like body was noticed. The caseose was precipitated from the suitably concentrated fluid with alcohol, boiled repeatedly with alcohol to free it from any adhering tyrosin, and finally dried at 110° C.

The substance was extremely soluble in water, the fluid remaining perfectly clear when heated. Acetic acid produced a heavy precipitate, soluble in excess, and in the acid fluid potassium ferrocyanide gave a heavy precipitate. Dilute nitric acid, added to the aqueous solution produced a white precipitate readily soluble in excess of acid, and when heated showed the xanthoprotein reaction.

Cupric sulphate also gave a heavy white precipitate. On analysis, the following results were obtained:

β deuterocaseose, formed by trypsin.

I. 0·3355 gram substance gave 0·1847 gram H_2O=6·11 per cent. H and 0·5990 gram CO_2=48·68 per cent. C.

II. 0·3459 gram substance gave 0·1871 gram H_2O=6·01 per cent. H and 0·6120 gram CO_2=48·24 per cent. C.

III. 0·5399 gram substance gave 63·0 c. c. N at 13·8° C. and 762·6 mm pressure = 13·63 per cent. N.

IV. 0·4166 gram substance gave by fusion with KOH+KNO_3 0·0300 gram $BaSO_4$=0·98 per cent. S; after deducting sulphur of ash = 0·95 per cent.

V. 0·5496 gram substance gave by fusion with KOH+KNO_3 0·0380 gram $BaSO_4$=0·95 per cent. S; after deducting sulphur of ash = 0·91 per cent.

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VI. 0·4804 gram substance gave 0·0365 gram = 9·59 per cent.

VII. 0·4272 gram substance gave 0·0405 gram ash = 9·43 per cent.

VIII. Ash from 0·8076 gram substance gave 0·0181 gram $BaSO_4$ = 0·03 per cent. S.

Percentage composition of ash-free substance.

						Average.
C	53·81	53·31	----	----	----	*53·56*
H	6·76	6.64	----	----	----	*6·70*
N	----	----	15·07	----	----	*15·07*
S	----	----	----	0·95	0·91	*0·93*
O	----	----	----	----	----	*23·74*
						100·00

This sample of β deutero shows very close agreement in composition and reactions with the like body formed by dilute sulphuric acid; both being characterized by the reaction with acetic acid and showing, by their reactions and composition, a closer relationship to the proto-like bodies than their non-precipitation by ammonium sulphate would appear to warrant. In composition, however, this body shows a much smaller percentage of carbon than the caseose precipitated by acetic acid.

For separation of the other caseoses formed in this digestion, the first ammonium sulphate precipitate was dissolved in water and protocaseose precipitated by saturation of the solution with sodium chloride. The precipitate, which was not very heavy, was purified by reprecipitation and dialysis. On dissolving the first salt precipitate in water quite a little residue was noticed, soluble in dilute sodium carbonate, but readily precipitated by the least trace of acid. The final neutral solution was concentrated, giving when heated a heavy coagulum which finally came together as a gummy mass. The clear fluid, separated from the gum, gave still another coagulum as the heating was continued. On cooling, the gummy matter readily dissolved. As the evaporation continued and the fluid became concentrated the gummy matter dissolved even in the hot fluid, and the caseose was finally precipitated while hot with alcohol, and the precipitate boiled repeatedly with alcohol for the complete removal of leucin and tyrosin.

In reactions, this body was apparently identical with protocaseose formed by pepsin-hydrochloric acid, except that with acetic acid and also with hydrochloric acid it yielded a heavy white precipitate, soluble in excess of acid. In water it dissolved almost completely, the solution, however, becoming turbid when heated and if sufficiently

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concentrated giving a gummy deposit as the heating continued. Further, the reaction with heat was the only respect in which this body differed from the preceding ammonium sulphate precipitate. Owing to insufficient quantity it was not analyzed.

In the filtrate from the first sodium chloride precipitate, a second caseose was precipitated by addition of a little 30 per cent. acetic acid. At first it separated as a flocculent precipitate, but on standing changed to a gummy mass, which dissolved more or less readily in water, and completely so when a little sodium carbonate was added to neutral reaction. After reprecipitation, the aqueous solution of the substance was neutralized, dialyzed, the solution concentrated without separation of any gummy matter, and the substance finally precipitated with alcohol. The precipitate was boiled repeatedly with alcohol and finally dried at 110° C. The dried substance was readily soluble in water and also in dilute acetic acid, potassium ferrocyanide producing in the latter solution a slight turbidity only. Added to an aqueous solution of the substance, acetic acid produced a heavy white precipitate readily soluble in excess of the acid. Nitric acid, likewise, produced a heavy white precipitate not so readily soluble in excess. Cupric sulphate also gave a heavy white precipitate. The aqueous solution of the caseose gave no turbidity whatever, when heated.

Analyzed it yielded the following results:

Acetic acid precipitate of caseose (a deuterocaseose?) formed by trypsin.

I. 0·5594 gram substance gave 0·3300 gram H_2O=6·55 per cent. H and 1·0924 grams CO_2=53·25 per cent. C.

II. 0·3670 gram substance gave 0·2157 gram H_2O=6·53 per cent. H and 0·7158 gram CO_2=53·19 per cent. C.

III. 0·3811 gram substance gave 44·4 c. c. N at 12·8° C. and 765·1 mm pressure =14·07 per cent. N.

IV. 0·2848 gram substance gave 33·0 c. c. N at 13·4° C. and 765·5 mm pressure =13·98 per cent. N.

V. 0·4351 gram substance gave 0·0230 gram ash =5·28 per cent.

VI. 0·4033 gram substance gave 0·0210 gram ash =5·26 per cent.

Percentage composition of ash-free substance.

					Average.
C	56·20	56·14	---	----	*56·17*
H	6·91	6·89	----	----	*6·90*
N	----	----	14·85	14·75	*14·80*

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It is thus obvious from the foregoing that by the action of trypsin, caseoses are formed of the same general nature as those formed by the action of pepsin-hydrochloric acid, and by hot dilute sulphuric acid, but with higher contents of carbon.

In other digestions with trypsin, made especially for the preparation of casein peptone, these caseoses were again separated and the foregoing reactions verified. The quantities, however, were too small to admit of their analysis.

III. *Casein peptone;*—from experiments by *Charles Norris, Jr., Ph.B.*

Supersaturation of a digestive fluid with ammonium sulphate, under proper conditions, suffices to entirely remove the preliminary products of proteolytic action. Proper conditions, however, are not obtained by simply adding the ammonium salt to a cold fluid, for as has been already pointed out an additional precipitate of proteose can nearly always be obtained, by heating the cold saturated solution until a thick crust of the ammonium salt forms on the hot fluid. In the present state of knowledge, we assume as peptone any amorphous product of proteolytic action precipitable by alcohol, and not precipitable by heating with ammonium sulphate added to saturation. Unquestionably, the albumose precipitated only by long boiling of a saturated ammonium sulphate solution, is much nearer to true peptone than those bodies more easily precipitated, but at present we are not inclined to accept as true peptone any body precipitable by ammonium sulphate under any conditions whatever. In the present series of experiments we have aimed to prepare a casein peptone, by the action of trypsin, entirely free from albumose in order to study its composition and reactions.

In the digestion of casein with trypsin, described in the preceding section, the filtrate from the ammonium sulphate saturation was heated for some time and the slight gummy film of caseose separated, after which as much of the ammonium sulphate as possible was removed by alternate crystallization, treatment with alcohol, etc. The last traces of the ammonium salt were removed by long continued dialysis in running water, and when finally the fluid gave no reaction with barium chloride it was evaporated to a syrup and precipitated with alcohol. The precipitated peptone was freed from any adhering tyrosin and leucin by repeated treatment with boiling alcohol, and finally dried at 110° C. until of constant weight. This proved a long operation. The peptone was so exceedingly hygroscopic and held on so tenaciously to the water, that it was only after

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long-continued drying that a constant weight was reached and then the odor was strongly suggestive of partial dissociation. In a previous article* on peptone, attention was called to the peculiar odor of valerianic acid almost invariably noticed when the fibrin peptone was dried at 110° C., but in that case it was found impossible to bring the product to a constant weight. With casein peptone, the same odor was noticeable on drying the product for analysis, but after a few days heating at 110° C., the weight of the product remained fairly constant.

On analysis, the casein peptone yielded the following results:

Casein antipeptone A.

I. 0·5792 gram substance gave 0·3185 gram H_2O=6·11 per cent. H and 0·9951 gram CO_2=46·85 per cent. C.

II. 0·4692 gram substance gave 0·2562 gram H_2O=6·07 per cent. H and 0·8060 gram CO_2=46·84 per cent C.

III. 0·4357 gram substance gave 55·5 c. c. N at 14·0° C. and 762·0 mm pressure=15·27 per cent. N.

IV. 0·6534 gram substance gave 83·0 c. c. N at 14·0° C. and 761·0 mm pressure=15·18 per cent. N.

V. 0·9703 gram substance gave 0·0656 gram ash=6·76 per cent.

VI. 0·4939 gram substance gave 0·0326 gram ash=6·60 per cent.

VII. 0·7032 gram substance gave by fusion with KOH+KNO_3 0·0386 gram $BaSO_4$=0·75 per cent. S.

VIII. 0·6526 gram substance gave by fusion with KOH+KNO_3 0·0337 gram $BaSO_4$=0·71 per cent. S.†

Percentage composition of ash-free substance.

							Average.
C	49·94	49·93	....	....	....	....	*49·94*
H	6·51	6·50	....	....	....	....	*6·51*
N	....	....	16·35	16·26	....	....	*16·30*
S	....	....	....	....	0·70	0·66	*0·68*
O	....	....	....	....	....	....	*26·57*
							100·00

Somewhat to our surprise, on testing the purified and dried peptone, we found that its aqueous solution gave a heavy white precipitate with nitric acid, soluble in excess of acid; likewise, a heavy precipitate with acetic acid, also soluble in excess; with cupric sul-

* Kühne and Chittenden, Peptone. Studies, vol. ii.

† The ash contained only a slight unweighable trace of sulphate.

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phate a heavy precipitate; and on saturation with ammonium sulphate in the cold an abundant gummy precipitate, the latter plainly indicating the presence of caseose. As the product originally gave no precipitate with ammonium sulphate, it would apparently follow that the peptone by long drying at 110° C. had been, in part, reconverted into caseose, and as the solution gave no turbidity by heat it would imply that the caseose formed by the reconversion of the peptone was the deutero body. Results similar to these were obtained by Kühne and Chittenden with fibrin peptone.

In a second digestion of trypsin, 1 kilo. of moist casein was warmed at 40° C. with 4 litres of 0·6 per cent. sodium carbonate solution containing trypsin, well thymolized, for one week. On the third day, the gelatinous coagulum already described made its appearance, but gradually disappeared and at the end of the week there was only a very small residue remaining. The neutralized and concentrated digestive fluid, freed from more or less tyrosin by cooling and crystallization, was in this case treated with rock salt to saturation, yielding, however, only a small precipitate of proto- and heterocaseose, which on purification agreed in reactions with the caseoses previously described. Addition of salt-saturated acetic acid to the sodium chloride filtrate from the foregoing caseoses, failed to give any precipitate whatever, and as the saturation of the fluid with ammonium sulphate gave only a slight gummy precipitate it is evident that in this digestion the casein was almost completely converted into peptone. In order to be quite sure of the complete removal of everything precipitable by the ammonium salt the mixture was boiled for some time with an excess of ammonium sulphate, and the filtrate treated as described under peptone *A* for the complete removal of tyrosin and ammonium sulphate.

The final product was exceedingly gummy and parted with the last traces of adhering alcohol very slowly. In fact, we found it best to dissolve the final alcoholic precipitate of peptone in a little water, and to drive off the alcohol from the solution by heat, after which the fluid was evaporated and the gummy peptone finally transformed into a friable mass by drying on a water-bath, and at last completely dried at 110° C. After its final precipitation with alcohol, an aqueous solution of the peptone gave no precipitate whatever, with nitric or acetic acid, neither with acetic acid and potassium ferrocyanide nor with cupric sulphate, or at the most nothing more than a faint turbidity. After being dried at 110° C. until of constant weight, the product then gave a decided gummy precipitate by saturation

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of its aqueous solution with ammonium sulphate, and like peptone *A* gave precipitates with nitric and acetic acid and with cupric sulphate.

On analysis the following results were obtained:

Casein antipeptone B.

I. 0·3770 gram substance gave 0·2023 gram H_2O=5·96 per cent. H and 0·6535 gram CO_2=47·25 per cent. C.

II. 0·2687 gram substance gave 0·1442 gram H_2O=5·96 per cent. H and 0·4667 gram CO_2=47·36 per cent. C.

III. 0·3732 gram substance gave 44·9 c. c. N at 14·4° C. and 757·0 mm pressure=14·31 per cent. N.

IV. 0·3801 gram substance gave 46·0 c. c. N at 14·0° C. and 756·0 mm pressure=14·38 per cent. N.

V. 0·4760 gram substance gave 0·0374 gram ash=7·86 per cent.

VI. 0·5396 gram substance gave 0·0429 gram ash=7·95 per cent.

Percentage composition of ash-free substance.

					Average.
C	51·35	51·42	----	----	*51·38*
H	6·47	6·47	----	----	*6·47*
N	----	----	15·54	15·61	*15·57*

In a third digestion with trypsin, 2 kilos. of casein were warmed at 40° C. for five days with 3 litres of 0·5 per cent. sodium carbonate containing an active trypsin solution, well thymolized. At the end of the second day, considerable casein antialbumid separated from the solution, this time more as a gummy precipitate than as a gelatinous coagulum. On the sixth day, the alkaline fluid was filtered from the small undigested residue, neutralized, concentrated, and the caseoses precipitated by saturation with ammonium sulphate. In this digestion, there was present only a very small trace of caseose precipitable by saturation with salt, but considerable precipitable by salt-saturated acetic acid. After repeated boiling of the ammonium sulphate-saturated fluid, for complete removal of caseoses, the peptone remaining was separated, purified and dried as already described. The product dried at 110° C. gave on analysis the following results.

Casein antipeptone C.

I. 0·3104 gram substance gave 0·1720 gram H_2O=6·15 per cent. H and 0·5252 gram CO_2=46·14 per cent. C.

II. 0·2756 gram substance gave 0·1513 gram H_2O=6·10 per cent. H and 0·4638 gram CO_2=45·89 per cent. C.

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III. 0·5432 gram substance gave 68·0 c. c. N at 14·6° C. and 756·4 mm pressure=14·88 per cent. N.

IV. 0·6280 gram substance gave 0·0449 gram ash=7·15 per cent.

V. 0·4060 gram substance gave 0·0288 gram ash=7·09 per cent.

Percentage composition of ash-free substance.

				Average.
C	49·64	49·40	----	49·52
H	6·63	6·57	----	6·60
N	----	----	15·99	15·99

This peptone, like the preceding, after being dried at 110° C. gave a small gummy precipitate on saturation of its solution with ammonium sulphate, and also gave a precipitate with nitric and acetic acid, and a slight turbidity with cupric sulphate.

In composition, all three of the peptones show a smaller percentage of carbon than the caseoses formed by trypsin, but somewhat to our surprise the percentage of carbon is higher than in some of the caseoses formed by pepsin-hydrochloric acid. The nature of the substance, however, which at present affords but little proof that we have to deal with a single body, the extreme difficulty of obtaining it in a condition of dryness suitable for analysis, and the almost utter impossibility of freeing it from adhering inorganic salts, all tend to throw doubt on the analytical data as expressing the composition of pure casein peptone. As already stated, there is unquestionably more or less of a decomposition or change attending the drying of the peptone. So pronounced is the hygroscopic character of these bodies, that when partially dried they will gain weight over strong sulphuric acid, and on being taken from the air bath while drying at 110° C. we often noticed on damp days a hissing noise as if from the rapid absorption of water. Further, as the drying progressed the odor of valerianic acid became quite pronounced and on testing the dried product, it was often found to have an acid reaction so pronounced in many cases as to give a sour taste to the peptone, in addition to the characteristic bitter. Before drying, the peptones were usually found to have a neutral reaction. These facts coupled with the changed behavior of the product towards ammonium sulphate, point to a change in the nature of the peptone, which may well be assumed to affect its composition, and hence we would have our analytical figures taken with some allowance.

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We also prepared a peptone, entirely non-precipitable by saturation with ammonium sulphate, by boiling 20 grams of pure deuterocaseose with 500 c. c. of 3 per cent. sulphuric acid for 14 hours. This preparation, after purification, was too small in quantity for analysis, but in reactions it showed close agreement with the antipeptones prior to their long drying at 110° C., viz: non-precipitation by acetic acid and potassium ferrocyanide, by nitric acid, by cupric sulphate, and by saturation with ammonium sulphate both in neutral and in acid solutions.

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Some Experiments on the Influence of Arsenic and Antimony on Glycogenic Function and Fatty Degeneration of the Liver. By R. H. Chittenden, and J. A. Blake, B.A., Ph.B.

Saikowsky's* oft-quoted experiments on rabbits with antimonic and arsenious acids have made clear that in both arsenical and antimonial poisoning there is pronounced fatty degeneration of the liver, with a lessening of the hepatic glycogen and in some cases even a total disappearance of it. With antimonic acid, Saikowsky found in his original experiments, that one-half to one gram of antimonic acid or other preparation of antimony per day, for fourteen or nineteen days in succession, gave rise to a fatty degeneration embracing the liver, kidneys, and even the heart. This has been verified by the experiments of Grohe and Mosler, who also state that in the duchy of Brunswick the peasantry give to the geese, when producing the famous fatty livers, a certain quantity of the white oxide of antimony every day.† With arsenic, Saikowsky likewise found that when rabbits are poisoned by a small dose so as to live from three to six days, the liver becomes much enlarged and very fatty and the glycogenic function nearly or quite abolished.

It is very evident, therefore, that in large quantities both arsenic and antimony have a special action on tissue changes, particularly on the liver. In the experiments referred to above, the quantities of poison given were quite large and with arsenic, particularly, their administration was soon followed by death. As neither of these substances are ordinarily used in medicine for an acute effect, it seemed to us of interest to study the action of small doses on the tissue changes of the liver, with a view to ascertaining whether non-toxic doses of these two poisons would produce a similar effect. It is ordinarily stated that in poisoning with antimony, phosphorus, and arsenic the nitrogenous products of tissue waste appear in the urine in much larger quantity than normally, owing to the increased decomposition which is going on.‡ Experiments of our own, however, have shown that

* Virchow's Archives, Band xxxiv, p. 78.

† Quoted from H. C. Wood's Therapeutics, p. 161.

‡ Brunton's Pharmacology, Therapeutics and Materia Medica, p. 360.

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small repeated doses of antimonious oxide are without influence on the excretion of nitrogen, sulphur, and phosphorus, and that hence when taken in non-toxic doses it has no noticeable action on proteid metabolism.* Without doubt, toxic doses do materially affect the nutrition of the body, but with a dog of 13 kilos. weight the administration of repeated doses of antimonious oxide, to the extent of 17 grains in 13 days, led to no apparent change in the amount of nitrogen etc. excreted, although the presence in the 24 hours' urine of 13–23 milligrams of antimony (Sb) gave evidence of decided absorption.

We have therefore tried a few experiments on rabbits and fowls to see what effect small repeated doses of arsenic and antimony would have on the liver, as indicated by its content of fat, glycogen, and sugar. The experiments were made in pairs, in which one animal of each pair served as a control for comparison, while the other, kept under the same conditions of diet, etc. so far as possible, was fed each day with arsenic or antimony as the case might be. At the end of the period both animals were killed and the livers analyzed. Naturally, the animals of each pair were of the same age, from the same brood, and so far as possible of the same body weight. During the experiment they were kept on a weighed diet of cracked corn, meal, etc., and were confined in suitable cages.

The methods of analysis were as follows: after determining the body weight, the liver was quickly removed, weighed and sampled by chopping, 10 grams or thereabouts were then weighed out accurately, dried on a water bath, ground to a fine powder and extracted in a fat extractor with warm ether until the fat was entirely removed.

For glycogen and sugar, 20–40 grams of the sampled liver were thoroughly extracted with hot water, (continuous extraction with water, frequently renewed, for several days) the extracts united, concentrated to a very small volume and precipitated with a large excess of 95 per cent. alcohol. The precipitate of glycogen, etc., was washed with alcohol, dissolved in a small volume of cold water to 100 c. c., sufficient hydrochloric acid added to make the fluid contain 2 per cent. HCl and heated on a water-bath for 15 hours. After neutralization, the volume was made up to 200 c. c. and the sugar determined in 25 c. c. of the fluid by Allihn's gravimetric method, and the glycogen calculated therefrom. The liver sugar was determined by evaporating the alcoholic fluid from the glycogen precipitate, dissolving the residue in a little water, adding sufficient sulphuric acid to make the mix-

* Chittenden and Blake. Studies from Laboratory of Physiological Chemistry, Yale University, vol. ii, p. 94.

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ture contain 2 per cent. H_2SO_4, boiling for two hours to convert the sugar wholly into dextrose, and then, after neutralization of the acid fluid and diluting to 200 c. c., testing its reducing power by Allihn's method.

Experiment I.

Action of arsenic on a fowl.

Period of dosing.

May 20–24,	0·1 grain As_2O_3 daily.	
" 25–31,	0·2 " " "	
June 1–15,	0·3 " " "	
	6·4 grains.	

	A, control fowl.	*B, arsenic fowl.*
Body weight May 20,	1814 grams.	1814 grams.
" " June 16,	1871 "	1686 "
	+57	−128
Weight of liver, June 16,	28·378 grams.	34·635 grams.

The liver of B, showed unmistakeable signs of fatty degeneration.

Determination of fat in liver.

A. control,	9·210 grams liver gave 0·3347 gram fat,	=	3·63 per cent.
B. arsenic,	13·848 " " " 1·8561 " "	=	13·40 "
			+9·77 per cent.

Determination of glycogen and sugar.

	A.	*B.*
Weight of liver used,	20·167 grams.	20·787 grams.

Glycogen A, control.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
25 c. c.	0·0744 gram.	0·0380 gram.	0·0342 gram.	0·2736 gram.	1·35
25	0·0727	0·0371	0·0334	0·2672	1·32

Glycogen B, arsenic.

25 c. c.	0·1720 gram.	0·0879 gram.	0·0791 gram.	0·6328 gram.	3·04
25	0·1713	0·0875	0·0787	0·6296	3·02

Sugar A, control.

25 c. c.	0·0299 gram.	0·0159 gram.	-----	0·1272 gram.	0·63
25	0·0332	0·0176	-----	0·1408	0·69

Sugar B, arsenic.

25 c. c.	0·0185 gram.	0·0102 gram.	-----	0·0816 gram.	0·39
25	0·0162	0·0091	-----	0·0728	0·35

100 grams of breast muscle from *B* gave 0·4 milligram of *As.*

In this experiment, then, we have as the apparent result of the long-continued feeding of arsenic a loss of body weight, a decided increase

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in the weight of the liver, a large increase in the liver fat, a gain in the amount of liver glycogen, and a loss in liver sugar.

Experiment II.

Action of arsenic on a fowl.

Period of dosing.

May 20–24,	0·1 grain	As_2O_3 daily.
" 25–31,	0·2 "	" "
June 1–15,	0·3 "	" "
" 16–18,	0·4 "	" "
	7·6 grains.	

Towards the end of the experiment the arsenic fowl appeared to be suffering somewhat from the toxic action of the poison, consequently the arsenic was discontinued after the 18th, but the fowl died on the 21st, and was immediately analyzed.

	A, control fowl.	*B, arsenic fowl.*
Body weight May 20,	1644 grams.	1531 grams.
" " June 21,	1757 "	1247 "
	+113	−284
Weight of liver, June 21,	55·830 grams.	33·729 grams.

Determination of fat in liver.

A, control,	13·740 grams liver gave 0·7462 gram fat,	=	5·40 per cent.
B, arsenic,	10·179 " " " 0·4925 " "	=	4·83 "
			−0·57 per cent.

Determination of glycogen and sugar.

	A.	*B.*
Weight of liver used,	42·090 grams.	23·550 grams.

Glycogen A, control.

Volume used.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
25 c. c.	0·5213 gram.	0·2500 gram.	0·2250 gram.	1·8000 gram.	4·27
25	0·5228	0·2504	0·2253	1·8024	4·28

Sugar A, control.

25 c. c.	0·0359 gram.	0·0188 gram.	-----	0·1504 gram.	0·35
25	0·0379	0·0198	-----	0·1584	0·37

In *B*, no trace of a reduction could be obtained for glycogen and only an unweighable trace for sugar.

In this experiment, therefore, we find a decided loss in body weight under the influence of the arsenic, a loss in the weight of the liver, a slight diminution in the liver fat and nearly a complete disappearance of both glycogen and sugar.

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Experiment III.

Action of arsenic on a rabbit.

Period of dosing.

May 25–31,	0·2 grain	As_2O_3 daily.
June 1–15,	0·3 "	" "
" 16–23,	0·4 "	" "
	9·1 grains.	

	A, control rabbit.	*B, arsenic rabbit.*
Body weight May 25,	1644 grams.	1502 grams.
" " June 24,	1616 "	1586 "
	−28	+84
Weight of liver June 24,	61·360 grams.	46·760 grams.

Determination of fat in liver.

A, control,	15·320 grams liver gave 0·4126 gram fat,	=	2·69 per cent.
B, arsenic,	14·435 " " " 0·2888 " "	=	2·00 "
			−0·69 per cent.

Determination of glycogen and sugar.

	A.	*B.*
Weight of liver used,	45·940 grams.	32·325 grams.

Glycogen A, control.*

Volume used for reduction.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
25 c. c.	0·2036 gram.	0·1045 gram.	0·0940 gram.	1·8800 grams.	4·13
25	0·2027	0·1041	0·0937	1·8740	4·07
		Glycogen B, arsenic.			
25 c. c.	0·5230 gram.	0·2503 gram.	0·2252 gram.	1·8016 grams.	5·57
25	0·5223	0·2500	0·2250	1·8000	5·54
		Sugar A, control.			
25 c. c.	0·0177 gram.	0·0098 gram.	-----	0·0784 gram.	0·17
25	0·0173	0·0097	-----	0·0776	0·16
		Sugar B, arsenic.			
25 c. c.	0·0254 gram.	0·0137 gram.	-----	0·1096 gram.	0·34
25	0·0232	0·0126	-----	0·1008	0·31

100 grams of muscle tissue yielded 0·3 milligram of *As.*

Here, we find under the influence of the arsenic, an apparent gain, though slight, in body weight, an evident diminution in the weight of the liver, together with a diminished percentage of liver fat. Apparently, however, the amount of liver glycogen and sugar are somewhat increased. The liver showed no approach to fatty degeneration on microscopic examination.

* The glycogen solution after boiling with acid, etc., was made up to 500 c. c. instead of 200 c. c.

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Experiment IV.

Action of antimony on a fowl.

Period of dosing.

May 20–24,	0·2 grain Sb_2O_3	daily.
" 25–31,	0·4 " "	"
June 1–7,	0·6 " "	"
" 8–14,	0·8 " "	"
	13·6 grains.	

	A, control fowl.	*B, antimony fowl.*
Body weight May 20,	1559 grams,	1616 grams,
" " June 15,	1531 "	1828 "
	−28	+212
Weight of liver June 15,	22·995 grams.	31·799 grams.

Determination of fat in liver.

A, control,	7·985 grams liver gave 0·4600 gram fat,	=	5·76 per cent.
B, antimony,	11·409 " " " 0·6741 " "	=	5·91 "
			+0·15 per cent.

Determination of glycogen and Sugar.

	A.	*B.*
Weight of liver used,	15·010 grams.	20·390 grams.

Glycogen A, control.

Volume used for reduction.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
25 c. c.	0·0577 gram.	0·0296 gram.	0·0266 gram.	0·2128 gram.	1·41
25	0·0563	0·0289	0·0260	0·2080	1·38

Glycogen B, antimony.

25 c. c.	0·1011 gram.	0·0514 gram.	0·0462 gram.	0·3696 gram.	1·81
25	0·1015	0·0516	0·0464	0·3712	1·82

Sugar A, control.

25 c. c.	0·0198 gram.	0·0109 gram.	-----	0·0872 gram.	0·58
25	0·0211	0·0115	-----	0·0920	0·61

Sugar B, antimony.

25 c. c.	0·0300 gram.	0·0160 gram.	-----	0·1280 gram.	0·62
25	0·0277	0·0148	-----	0·1184	0·58

100 grams of breast muscle gave 1·2 milligrams Sb.

These results would seem to indicate that antimonious oxide tends to increase the body weight, and apparently also that of the liver. The liver fat is obviously not much affected in this experiment, while the glycogen shows a slight increase in the antimonial fowl.

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Experiment V.

Action of antimony on a fowl.

Period of dosing.

May 20–24,	0·2 grain Sb_2O_3 daily.
" 25–31,	0·4 " " "
June 1– 7,	0·6 " " "
" 8–15,	0·8 " " "
" 16–19,	1·0 " " "
	18·4 grains.

	A, control fowl.	*B, antimony fowl.*
Body weight May 20,	1701 grams.	1757 grams.
" " June 20,	1644 "	1814 "
	−57	+57
Weight of liver June 20,	34·177 grams.	35·440 grams.

Determination of fat in liver.

A, control,	9·477 grams liver gave	0·3890 gram fat,	=	4·10 per cent.
B, antimony,	9·579 " " "	0·5088 " "	=	5·31 "
				+1·21 per cent.

The appearance of the liver in *B* indicated fatty degeneration.

Determination of glycogen and sugar.

	A.	*B.*
Weight of liver used,	24·700 grams.	25·871 grams.

Glycogen A, control.

Volume used for reduction.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
25 c. c.	0·2253 gram.	0·1160 gram.	0·1044 gram.	0·8352 gram.	3·38
25	0·2253	0·1160	0·1044	0·8352	3·38

Glycogen B, antimony.

25 c. c.	0·2480 gram.	0·1281 gram.	0·1153 gram.	0·9224 gram.	3·56
25	0·2479	0·1280	0·1150	0·9200	3·55

Sugar A, control.

25 c. c.	0·0360 gram.	0·0189 gram.	-----	0·1512 gram.	0·61
25	0·0360	0·0189	-----	0·1512	0·61

Sugar B, antimony.

25 c. c.	0·0383 gram.	0·0200 gram.	-----	0·1600 gram.	0·62
25	0·0372	0·0195	-----	0·1560	0·60

100 grams of breast muscle gave 1·3 milligrams Sb.

In this experiment, there was pronounced fatty degeneration of the liver in the antimonial fowl. Further, as in the last experiment, there was apparently a slight increase both in body weight and in the weight of the liver.

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Corresponding with the fatty degeneration, there was found 1·2 per cent. more fat in the antimonial liver than in the control.

The liver glycogen was also slightly increased under the influence of the antimony.

Experiment VI.

Action of antimony on a rabbit.

Period of dosing.

May 25–31,	0·4 grain Sb_2O_3 daily.
June 1– 7,	0·6 " " "
" 8–15,	0·8 " " "
" 16–21,	1·0 " " "
	19·4 grains.

	A, control rabbit.	*B,* antimony rabbit.*
Body weight May 25,	2012 grams.	1814 grams.
" " June 23,	1998 "	1701 "
	−14	−113
Weight of liver June 23,	38·219 grams.	42·741 grams.

Determination of fat in liver.

A, control,	9·375 grams liver gave 0·3575 gram fat,	=	3·81 per cent.
B, antimony,	10·971 " " " 0·3432 " "	=	3·12 "
			−0·69 per cent.

Determination of glycogen and sugar.

	A.	*B.*
Weight of liver used,	28·844 grams.	31·770 grams.

Glycogen A, control.

Volume used for reduction.	Weight Cu.	Equivalent in dextrose.	Equivalent in glycogen.	Total amount.	Per cent.
25 c. c.	0·0431 gram.	0·0224 gram.	0·0201 gram.	0·1608 gram.	0·55
25	0·0441	0·0229	0·0206	0·1648	0·57

Sugar A, control.

25 c. c.	0·0445 gram.	0·0231 gram.	-----	0·1848 gram.	0·64
25	0·0440	0·0229	-----	0·1832	0·63

In *B*, there was no reduction for either glycogen or sugar.

100 grams of muscle gave 1·0 milligram Sb.

In this experiment, towards the end, the animal was evidently suffering from the toxic action of the antimony, and the body weight is seen to be noticeably diminished. The weight of the liver, however, was apparently increased by the antimony, although there was a

* The last two days the antimony rabbit ate very little. Hence, the oxide was not given after the 21st. The animal was evidently much affected by the antimony and its general nutrition was at the last very poor.

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diminution in the liver fat, and a complete disappearance of both glycogen and sugar.

While these experiments are far too few in number to generalize from, yet it would appear that small doses of antimonious oxide long continued tend to increase body weight, and particularly the weight of the liver. Further, the increase in liver weight is accompanied by an increased percentage of fat and a slight increase of liver glycogen.

With corresponding doses of arsenious oxide, on the other hand, the results would indicate a diminution in body weight, likewise a diminution in the weight of the liver and also in the amount of liver fat. As regards glycogen, two of the results show an increased amount in the arsenical livers, while in one experiment there was a total disappearance of glycogen. On the other hand, as Experiment No. I indicates, there may be, with small doses of arsenic, a very pronounced fatty degeneration of the liver, accompanied by an increased liver weight and an increase in the percentage of liver glycogen.

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The Nature and Chemical Composition of the Myosin of Muscle Tissue. By R. H. Chittenden and G. Wyckoff Cummins, Ph.D.

In spite of the interest attached to this peculiar proteid substance, little attention has been directed to its chemical nature since the time of its discovery by Kühne.* In fact, Danilewsky† is the only investigator who appears to have studied its chemical relations to any extent, and so far as we are aware no attempt has ever been made to ascertain its chemical composition. With this fact in mind, it has been the main object of the present investigation to study the chemical composition of pure myosin, and to determine the differences that may exist in the chemical nature, or in the properties, of myosin prepared from various animal sources. Unfortunately, our work was completed before Halliburton's‡ recent paper on muscle-plasma was published, otherwise we should have attempted to verify some of his interesting discoveries regarding the muscle clot, and possibly have modified somewhat the character of our work.

The researches of Kühne, Hoppe-Seyler,§ Weyl,‖ and Danilewsky have shown that myosin, both from the animal and vegetable kingdoms, is a globulin body, soluble in dilute sodium and ammonium chloride solutions, as well as in sulphate of magnesia and sulphates of the alkalies, and precipitable therefrom either by the addition of salt in substance or by dilution with much water. As stated by Danilewsky, myosin is most advantageously extracted from muscle tissue by ammonium chloride, in solutions of from 7–20 per cent. We have, likewise, found this to be the case by comparative tests, and have, therefore, in all of our preparations of myosin for analysis, used ammonium chloride as the extractive, preferably of 15 per cent. strength. For separation of myosin from the ammonium chloride

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* Kühne, Protoplasma, Leipzig, 1864. Also Lehrbuch der physiologische Chemie.

† Myosin, seine Darstellung, Eigenschaften, Umwandlung in Syntonin und Rückbildung aus demselben. Zeitschrift für physiologische chemie. Band v, p. 158.

‡ Journal of Physiology, vol. viii, p. 133.

§ Handbuch der Chem. Analyse, 4 Auflage, p. 236.

‖ Beitrage zur kenntniss thierischer und pflanzlicher eiweisskörper, Zeitschrift für physiologische Chemie, Band i, p. 72.

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solution we have mainly employed two methods, either dilution with a large volume of distilled water, or dialysis of the ammonium chloride solution until the salt is entirely removed. In this latter process, we have often observed that the separation of myosin partakes more of the nature of a coagulation than of an ordinary precipitation. A moderately strong ammonium chloride solution of myosin, on being placed in a parchment bag and suspended in running water, will ordinarily at the end of two or three days be converted into a semi-solid, jelly-like mass, which later on contracts more or less, but still shows all the characters of a genuine clot. In fact, we are inclined, with Halliburton, to consider this a genuine re-coagulation rather than a precipitation. We are inclined, however, to believe that separation of myosin by dialysis is hardly as satisfactory in the preparation of small quantities, as precipitation by water, since on dialysis the jellying of the myosin naturally tends to enclose some of the salt and also any other proteids possibly present in the solution, while by precipitation with water the myosin is flocculent, easily washed and thus more surely freed from both salt and albumin.

Myosin A, from ox muscle.

The first sample of myosin was prepared from a freshly killed ox. The finely chopped muscle from the thigh was freed, so far as possible, from all traces of blood and soluble albumin by long soaking and frequent kneading with water. The water was frequently changed and kept thoroughly thymolized so as to prevent any approach to putrefaction. When the washings failed to give any turbidity by heat, or by acetic acid and potassium ferrocyanide, the washed tissue was placed in 10 litres of a 15 per cent. solution of ammonium chloride for extraction of the myosin. The resultant opalescent fluid was filtered through paper and the myosin precipitated by saturation with sodium chloride. The precipitate was quickly strained off, dried somewhat between folds of filter paper, dissolved in a small amount of water and reprecipitated by treatment of the fluid with a large volume of water. The precipitate so obtained was washed with water until the washings gave no reaction for chlorides, when it was treated with weak alcohol and finally with 95 per cent. and absolute alcohol, and ether.

When partially dried, it was ground fine and further dried at 110° C. until of constant weight, for analysis.

Its composition is shown in the accompanying table.

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ANALYSIS OF MYOSIN A, FROM OX MUSCLE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ from the Ash. gram.	S of Ash. % of sub.	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	S after deducting S of Ash. %
I	0·3878	0·2442	6·99	0·7400	52·04	---	---	------	---	----	---	----	---	----	---	-----
II	0·5659	0·3579	7·03	1·0809	52·09	---	---	------	---	----	---	----	---	----	---	-----
III	0·5996	------	---	------	----	81·7	8·7	758·4	16·61	----	---	----	---	----	---	-----
IV	0·5783	------	---	------	----	80·1	10·0	757·7	16·69	----	---	----	---	----	---	-----
V	0·6794	------	---	------	----	---	---	------	---	0·0101	1·49	----	---	----	---	-----
VI	0·8173	------	---	------	----	---	---	------	---	0·0121	1·48	----	---	----	---	-----
VII	1·4967	------	---	------	----	---	---	------	---	----	---	0·0064	0·05	----	---	-----
VIII	0·6930	------	---	------	----	---	---	------	---	----	---	----	---	0·0799	1·58	1·53
IX	0·9133	------	---	------	----	---	---	------	---	----	---	----	---	0·0965	1·44	1·39

Percentage composition of the ash-free substance.

							Average.
C	52·83	52·84	----	----	----	----	*52·84*
H	7·10	7·13	----	----	----	----	*7·12*
N	----	----	16·84	16·94	----	----	*16·89*
S	----	----	----	----	1·56	1·42	*1·49*
O	----	----	----	----	----	----	*21·66*
							100·00

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Myosin B, from ox muscle.

A solution of myosin in 15 per cent. ammonium chloride was prepared from 4 kilos. of freshly killed beef, as described under A. The myosin was then separated from the solution by dialysis, continued until nearly all of the chloride was removed. The last traces of the ammonium salt were separated by filtration of the gelatinous myosin through chamois skin, and washing with water. The preparation was then treated exactly as A, and dried at 110° C. for analysis.

By long continued contact with water, as in dialysis, the precipitated or coagulated myosin is rendered insoluble in dilute salt solutions, as noticed by Weyl and others, hence in this preparation it was not possible to purify the substance by reprecipitation.

The composition of the product (see the accompanying table) is essentially the same as that of the preceding preparation.

Myosin C, from sheep's muscle.

This sample of myosin was obtained from fresh mutton in essentially the same manner as the preceding preparation, viz: by extraction of the thoroughly washed tissue with 15 per cent. ammonium chloride, and separation of the myosin by dialysis.

On analysis, it was found to possess a somewhat higher percentage of carbon than the two myosins from ox muscle, but in other respects it was identical with them.

Myosin D, from calf's muscle.

A solution of myosin was prepared from 3 kilos. of fresh, lean muscle from a young calf, by extraction of the thoroughly washed tissue with 15 per cent. ammonium chloride solution.

From one-half of the filtered fluid, myosin was precipitated by dilution with water and purified by washing with water until chlorides were entirely removed. It was then washed with weak alcohol, finally with absolute alcohol and ether, and then dried at 110° C. for analysis (D^I).

From the other half of the ammonium chloride solution, myosin was separated by addition of ammonium chloride in substance. The precipitate was freed from excess of ammonium chloride by addition of just enough water to dissolve the salt, after which the flocculent myosin was strained off, dissolved in a little water, and precipitated by pouring the solution into a large volume of water. It was

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ANALYSIS OF MYOSIN B, FROM OX MUSCLE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ from the Ash. gram.	S of Ash. % of sub.	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	S after deducting S of Ash. %
I	0·4306	0·2719	7·01	0·8237	52·16	---	---	------	---	----	---	----	---	----	---	-----
II	0·3932	0·2500	7·06	0·7515	52·12	---	---	------	---	----	---	----	---	----	---	-----
III	0.5061	------	---	------	----	67·8	9·8	764·6	16·34	----	---	----	---	----	---	-----
IV	0·6005	------	---	------	----	81·3	9·7	762·6	16·48	----	---	----	---	----	---	-----
V	1·0425	------	---	------	----	---	---	------	---	0·0072	0·69	----	---	----	---	-----
VI	0·6690	------	---	------	----	---	---	------	---	0·0046	0·69	----	---	----	---	-----
VII	1·7115	------	---	------	----	---	---	------	---	----	---	0·0046	0·05	----	---	-----
VIII	0·6277	------	---	------	----	---	---	------	---	----	---	----	---	0·0597	1·31	1·26
IX	0·6358	------	---	------	----	---	---	------	---	----	---	----	---	0·0594	1·29	1·24

Percentage composition of the ash-free substance.

							Average.
C	52·53	52·48	---	----	----	----	*52·51*
H	7·06	7·11	----	----	----	----	*7·09*
N	----	----	16·46	16·59	----	----	*16·53*
S	----	----	----	----	1·26	1·24	*1·25*
O	----	----	----	----	----	----	*22·62*
							100·00

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ANALYSIS OF MYOSIN C, FROM SHEEP'S MUSCLE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T.°C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ from the ash. gram.	S of Ash. % of sub.	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	S after deducting S of ash. %
I	0·4945	0·3150	7·08	0·9603	52·95	---	---	----	---	----	---	----	---	----	---	-----
II	0·3539	0·2255	7·08	0·6863	52·88	---	---	----	---	----	---	----	---	----	---	-----
III	0·4600	-----	----	----	----	61·8	10·2	761·1	16·29	----	---	----	---	----	---	-----
IV	0·7477	----	----	----	----	101·3	10·2	762·1	16·41	----	---	----	---	----	---	-----
V	0·5170	----	----	----	----	---	---	----	---	0·0031	0·60	----	---	----	---	-----
VI	0·8969	----	----	----	----	---	---	----	---	0·0051	0·57	----	---	----	---	-----
VII	1·4139	----	----	----	----	---	---	----	---	----	---	0·0037	0·036	----	---	-----
VIII	0·7761	----	----	----	----	---	---	----	---	----	---	----	---	0·0699	1·24	1·20
IX	0·7059	----	----	----	----	---	---	----	---	----	---	----	---	0·0667	1·29	1·25

Percentage composition of the ash-free substance.

							Average.
C	53·27	53·20	----	----	----	----	*53·24*
H	7·12	7·12	----	----	----	----	*7·12*
N	----	----	16·39	16·51	----	----	*16·45*
S	----	----	----	----	1·20	1·25	*1·23*
O	----	----	----	----	----	----	*21·96*
							100·00

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then washed with water, alcohol, and ether, and dried at 110° C. (D^{II}).

The composition of both products is shown in the accompanying tables.

From the analytical data it is seen that the two preparations show close agreement, although there are minor differences; D^{II}, for example, containing a slightly higher percentage of carbon and a correspondingly lower percentage of nitrogen than D^{I}. Further, D^{II} contains less than half as much ash as the other preparation. Both show fairly close agreement with the myosins from ox muscle, with perhaps a slightly higher average percentage of nitrogen.

Myosin E, from fish.

So far as we are aware, little attention has been paid to the myosin from fish flesh. Myosin is assumed to be present and is supposed to be of the same general nature as the myosin from other forms of muscle tissue.

We first tried the separation of myosin from fresh cod (*Gadus callarias* or *G. morrhua*), using 2 kilos. of the fresh, lean tissue obtained in market. The final ammonium chloride extract (15 per cent.) failed to give anything more than an insignificant precipitate, either by dilution with water or by saturation of the fluid with sodium chloride.

A second preparation was attempted from the flesh of the halibut (*Hippoglossus vulgaris*); 2·5 kilos. of fresh tissue, free from fasciæ, fat and integument were thoroughly extracted with thymolized water for several days, the chopped tissue being well rubbed up with the water to insure complete removal of soluble albumins, etc. The thoroughly washed fibre was then triturated with a 15 per cent. ammonium chloride solution and allowed to stand in contact with it for 24 hours. The filtered fluid gave a decided precipitate on boiling, and also on addition of salt to saturation.

Myosin was separated from the ammonium chloride solution by dialysis as a more or less gelatinous precipitate, and was prepared for analysis by washing with thymolized water, and treatment with alcohol and ether.

Dried at 110° C. until of constant weight, it yielded the following results:

I. 0·3335 gram substance gave 0·2043 gram H_2O = 6·08 per cent. H and 0·6257 gram CO_2 = 51·16 per cent. C.

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ANALYSIS OF MYOSIN D[I], FROM CALF'S MUSCLE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ from the ash. gram.	S of Ash. % of sub.	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	S after deducting S of ash. %
I	0·3541	0·2201	6·91	0·6646	51·18	---	---	----	---	----	---	----	---	----	- -	-----
II	0·3925	0·2439	6·93	0·7395	51·33	---	---	----	---	----	---	----	---	----	---	-----
III	0·6283	----	----	----	----	87·4	9·8	749·8	16·64	----	---	----	---	----	---	-----
IV	0·6065	----	----	----	----	82·6	10·7	768·1	16·64	----	---	----	---	----	---	-----
V	0·9821	----	-- -	--- -	----	---	---	----	---	0·0291	2·95	----	---	---	---	-----
VI	0·7361	----	----	----	----	---	---	----	---	0·0214	2·91	----	---	----	---	-----
VII	1·7182	----	----	----	----	---	---	----	---	----	---	0·0130	0·10	----	---	-----
VIII	0·6707	----	----	----	----	---	---	----	---	----	---	----	---	0·0679	1·39	1·29
IX	0·5690	----	----	----	----	---	---	----	---	----	---	----	---	0·0575	1·39	1·29

Percentage composition of the ash-free substance.

							Average.
C	52·79	52·88	----	----	----	----	*52·84*
H	7·11	7·11	----	----	----	----	*7·11*
N	----	----	17·14	17·14	----	----	*17·14*
S	----	----	----	----	1·33	1·33	*1·33*
O	----	----	----	----	----	----	*21·58*
							100·00

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ANALYSIS OF MYOSIN D[II], FROM CALF'S MUSCLE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure. mm.	N %	Ash found. gram.	Ash. %	$BaSO_4$ from the ash. gram.	S of Ash. % of sub.	$BaSO_4$ after fusion with $KOH+KNO_3$ gram.	S %	S after deducting S of ash. %
I	0·3284	0·2078	7·03	0·6803	52·34	---	---	------	---	----	---	----	---	----	---	----
II	0·2290	0·1457	7·07	0·4396	52·35	---	---	------	---	----	---	----	---	----	---	----
III	0·6012	------	---	------	----	82·8	11·0	768·1	16·81	----	---	----	---	----	---	----
IV	0·5682	------	---	------	----	78·5	11·4	762·5	16·71	----	---	----	---	----	---	----
V	0·5994	------	---	------	----	---	---	------	---	0·0070	1·17	----	---	----	---	----
VI	0·8031	------	---	------	----	---	---	------	---	0·0096	1·19	----	---	----	---	----
VII	1·4025	------	---	------	----	---	---	------	---	----	---	0·0037	0·036	----	---	----
VIII	0·5575	------	---	------	----	---	---	------	---	----	---	----	---	0·0521	1·28	1·24
IX	0·6235	------	---	------	----	---	---	------	---	----	---	----	---	0·0576	1·27	1·23

Percentage composition of the ash-free substance.

							Average.
C	52·97	52·97	----	----	----	----	*52·97*
H	7·11	7·15	----	----	----	----	*7·13*
N	----	----	17·01	16·91	----	----	*16·96*
S	----	----	----	----	1·26	1·24	*1·25*
O	----	----	----	----	----	----	*21·69*
							100·00

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ANALYSIS OF MYOSIN F[1], FROM OX MUSCLE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found.			N %	Ash found. gram.	Ash %	$BaSO_4$ from the ash. gram.	S of Ash. % of sub.	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	S after deducting S of ash. %
						c. c.	T. °C.	Pressure. mm.								
I	0·4229	0·2661	6·99	0·8109	52·29	---	---	------	---	----	---	----	---	----	---	-----
II	0·3655	0·2315	7·03	0·7019	52·36	---	---	------	---	----	---	----	---	----	---	-----
III	0·6159	------	---	------	---	84·3	10·1	754·3	16·45	----	--	----	---	----	---	-----
IV	0·6100	------	---	------	---	83·6	9·2	755·7	16·57	----	---	---	---	----	---	-----
V	0·7144	------	---	------	---	---	---	------	---	0·0088	1·23	----	---	----	---	-----
VI	0·7000	----	---	------	---	---	---	------	---	0·0090	1·29	----	---	----	---	-----
VII	1·4144	------	---	------	---	---	---	------	---	----	---	0·0050	0·05	----	---	-----
VIII	0·6112	------	---	------	---	---	---	------	---	----	---	----	---	0·0579	1·30	1·25
IX	0·5189	------	---	------	---	---	---	------	---	----	---	----	---	0·0529	1·40	1·35
X	0·5882	------	---	------	---	---	---	------	---	----	---	----	---	0·0545	1·27	1·22

Percentage composition of the ash-free substance.

								Average.
C	52·95	53·04	----	----	----	----	----	*52·99*
H	7·08	7·13	----	----	----	----	----	*7·11*
N	----	----	16·67	16·78	----	----	----	*16·73*
S	----	----	----	----	1·27	1·37	1·24	*1·29*
O	----	----	----	----	----	----	----	*21·88*
								100·00

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ANALYSIS OF MYOSIN F^{II}, FROM OX MUSCLE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure. mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ from the ash. gram.	S of Ash. % of sub.	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	S after deducting S of ash. %
I	0·3369	0·2113	7·00	0·6381	51·63	---	---	------	---	----	---	----	---	----	---	-----
II	0·4515	0·2804	6·92	0·8582	51·83	---	---	------	---	----	---	----	---	----	---	-----
III	0·6228	------	---	------	---	84·4	10·8	758·0	16·33	----	---	----	---	----	---	-----
IV	0·4050	------	---	------	---	55·5	10·1	754·2	16·48	----	---	----	---	----	---	-----
V	0.5905	------	---	------	---	---	---	------	---	0·0123	2·08	----	---	----	---	-----
VI	0·7229	------	---	------	---	---	---	------	---	0·0146	2·02	----	---	----	---	-----
VII	1·3134	------	---	------	---	---	---	------	---	----	---	0·0074	0·077	----	---	-----
VIII	0·5634	----	---	------	---	---	---	------	---	----	---	----	---	0·0565	1·38	1·30
IX	0·7243	------	---	------	---	---	---	------	---	----	---	----	---	0·0705	1·32	1·24

Precentage composition of the ash-free substance.

							Average.
C	52·73	52·92	----	----	----	----	*52·83*
H	7·14	7·05	----	----	----	----	*7·10*
N	----	----	16·67	16·81	----	----	*16·74*
S	----	----	----	----	1·33	1·28	*1·31*
O	----	----	----	----	----	----	*22·02*
							100·00

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II. 0·4895 gram substance gave 67·8^{cc} N at 15·4° C. and 761·6^{mm} pressure = 16·50 per cent. N.

III. 0·4939 gram substance gave 0·0116 gram ash = 2·35 per cent.

Percentage composition of ash-free substance,

C 52·39, H 6·97, N 16·74.

The amount of myosin obtained from the fish muscle was not at all commensurate with the amount of flesh experimented with. Danilewsky,* however, found in the muscle tissue of the perch 8·56 per cent. of myosin as contrasted with 7·5 per cent. in ox muscle. Whether our inability to separate a corresponding amount from the tissue of the cod and halibut was due to the character of the tissue, to its possible staleness, or to inferior methods of separation we cannot say. Certainly, the yield of myosin in our case was not as great as from the muscle tissue of the ox, calf, lamb or sheep.

In composition, and in such reactions as we were able to try, the fish myosin was not unlike the myosin from other preparations.

Myosin F, from ox muscle.

A solution in 15 per cent. ammonium chloride was prepared as in preceding cases and the myosin precipitated by addition of a large amount of water.

One half of the precipitated myosin was washed with water until all ammonium chloride was removed, then with alcohol and ether, and dried for analysis (F^{I}).

The other half of the myosin was washed somewhat with water, not enough to render it insoluble, then dissolved in 10 per cent. sodium chloride solution, reprecipitated by dilution with water, thoroughly washed and dried (F^{II}).

The composition of the two products is shown in the accompanying tables. The results show close agreement with each other, and with the preceding preparations.

Myosin G, from ox muscle.

According to Danilewsky, a 15 per cent. solution of ammonium chloride is stronger than necessary for the extraction of myosin from

* Ueber die Abhänghigkeit der contractionsart der muskeln von der mengen verhältnissen einiger ihrer Bestandtheile. Zeitschrift für physiologische Chemie. Band vii, p. 124.

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muscle tissue. In fact, microscopic examination showed that 15 per cent. solutions of this salt generally cause destruction of the muscle fibres, while weaker solutions of the ammonium salt, as 5 per cent., extract the myosin equally well, without injuring the structural elements in the least.

Thinking that possibly a purer myosin might be obtained by the use of a weaker salt solution, several kilos. of thoroughly washed muscle tissue were rubbed up with an excess of 5 per cent. ammonium chloride and kept in contact with it for 48 hours. From the filtered solution, myosin was separated by addition of a large volume of water and the product washed and dried for analysis (G^{I}).

The tissue remaining after extraction as described, was treated with a fresh 5 per cent. solution of ammonium chloride, and from this fluid a second precipitate of myosin was obtained by addition of water. This also was washed and dried for analysis (G^{II}).

The residue of tissue still remaining was then extracted with a 15 per cent. solution of ammonium chloride. The extract so obtained gave a flocculent precipitate on saturation with sodium chloride, and a coagulum appeared on boiling the solution, but no precipitate could be obtained on addition of a large volume of water. It is thus evident that there was an almost complete extraction of myosin by the 5 per cent. solution of the ammonium salt.

On precipitating the small amount of myosin present in the 15 per cent. ammonium chloride solution by saturation of the fluid with salt, a clear filtrate was obtained which gave a turbidity on boiling and also on addition of acetic acid and potassium ferrocyanide, thus showing the presence of a small amount of soluble albumin.

The composition of the two myosins, as seen from the accompanying tables, does not differ at all from that of other preparations.

Myosin H, from muscle of sheep.

In this preparation, the thoroughly washed and chopped muscle was partially extracted with a 5 per cent. ammonium chloride solution, and the myosin separated by addition of water. It was then washed and dried for analysis (H^{I}).

The residue of tissue was extracted with a 15 per cent. solution of ammonium chloride and the myosin separated from this solution, likewise, by addition of water (H^{II}).

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ANALYSIS OF MYOSIN G[I], FROM OX MUSCLE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure. mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ from the ash. gram.	S of Ash. % of sub.	$BaSO_4$ after fusion with $KOH+KNO_3$ gram.	S %	S after deducting S of ash. %
I	0·3877	0·2482	7·11	0·7487	52·31	...	...	.....	...	....	...	....	...	....	...	.....
II	0·4690	0·2974	7·05	0·8979	52·21	...	...	.....	...	....	...	....	...	....	...	.....
III	0·6343	.....	...	.....	...	85·5	12·0	761·8	16·24	....	...	....	...	....	...	.....
IV	0.5718	.....	...	.....	...	77·6	13·6	765·6	16·32	....	...	....	...	....	...	.....
V	0·4814	.....	...	.....	...	...	...	.....	...	0·0071	1·47	....	...	....	...	.....
VI	0·7234	.....	...	.....	...	...	...	.....	...	0·0109	1·51	....	...	....	...	.....
VII	1·2048	.....	...	.....	...	...	...	.....	...	....	...	0·0071	0·08	....	...	.....
VIII	0·5281	.....	...	.....	...	...	...	.....	...	....	...	....	...	0·0484	1·26	1·18
IX	0·5719	.....	...	.....	...	...	...	.....	...	...	...	....	...	0·0506	1·22	1·14

Percentage composition of the ash-free substance.

							Average.
C	53·09	53·00	....	....	....	....	*53·05*
H	7·22	7·16	....	....	....	....	*7·19*
N	....	....	16·47	16·57	....	....	*16·52*
S	....	....	....	....	1·20	1·16	*1·18*
O	....	....	....	....	....	....	*22·06*
							100·00

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ANALYSIS OF MYOSIN G^{II}, FROM OX MUSCLE.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure. mm.	N %	Ash found. gram.	Ash %	$BaSO_4$ from the Ash. gram.	S of Ash. % of sub.	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	S after deducting S of Ash. %
I	0·4788	0·3021	7·01	0·9077	51·68	---	---	------	---	----	---	----	---	----	----	-----
II	0·3802	0·2361	6·90	0·7221	51·78	---	---	------	---	----	---	----	---	----	----	-----
III	0·8286	------	---	------	---	113·3	13·6	765·8	16·43	----	---	----	---	----	----	-----
IV	0·4869	------	---	------	---	66·5	14·1	768·4	16·49	----	---	----	---	----	----	-----
V	0·5740	------	---	------	---	---	---	------	---	0·0121	2·11	----	---	----	----	-----
VI	0·5348	------	---	------	---	---	---	------	---	0·0105	1·96	----	---	----	----	-----
VII	1·0088	------	---	------	---	---	---	------	---	----	---	0·0055	0·075	----	----	-----
VIII	0·5490	------	---	------	---	---	---	------	---	----	---	----	---	0·0488	1·22	1·14
IX	0·5918	------	---	------	---	---	---	------	---	----	---	----	---	0·0517	1·20	1·12

Percentage composition of the ash-free substance.

							Average.
C	52·77	52·86	----	----	----	----	*52·82*
H	7·16	7·05	----	----	----	----	*7·11*
N	----	----	16·78	16·83	----	----	*16·80*
S	----	----	----	----	1·17	1·15	*1·16*
O	----	----	----	----	----	----	*22·11*
							100·00

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ANALYSIS OF MYOSIN H[1], FROM MUSCLE OF SHEEP.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. °C.	N found. Pressure. mm.	N %	Ash found. gram.	Ash. %	$BaSO_4$ from the Ash. gram.	S of Ash. % of sub.	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	S after deducting S of Ash. %
I	0·3904	0·2441	6·95	0·7440	51·96	...	...	.....	...	....	...	.....	...	....	...	....
II	0·4537	0·2854	7·01	0·8637	51·94	...	...	.....	...	....	...	.....	...	....	...	....
III	0·2960	.....	...	.....	...	40·9	14·3	766·4	16·63	....	...	.....	...	....	...	....
IV	0·5605	.....	...	.....	...	...	...	.....	...	0·0096	1·71	.....	...	....	...	....
V	0·6490	.....	...	.....	...	...	...	.....	...	0·0108	1·67	.....	...	....	...	....
VI	1·2095	.....	...	.....	...	...	...	.....	...	....	...	0·0084	0·04	....	...	....
VII	0·5423	.....	...	.....	...	...	...	.....	...	....	...	.....	...	0·0464	1·18	1·14
VIII	0·5589	.....	...	.....	...	...	...	.....	...	....	...	.....	...	0·0553	1·35	1·31
IX	0·5233	.....	...	.....	...	...	...	.....	...	....	...	.....	...	0·0526	1·38	1·34

Percentage composition of the ash-free substance.

							Average.
C	52·86	52·82	....	....	....	....	*52·84*
H	7·07	7·13	....	....	....	....	*7·10*
N	....	....	16·91	....	....	....	*16·91*
S	....	....	....	1·15	1·32	1·36	*1·28*
O	....	....	....	....	....	....	*21·87*
							100·00

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ANALYSIS OF MYOSIN H^{II}, FROM MUSCLE OF SHEEP.

No.	Substance used. gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c. c.	N found. T. ° C.	N found. Pressure. mm.	N %	Ash. found. gram.	Ash. %	$BaSO_4$ from the Ash. gram.	S of Ash. % of sub.	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	S after deducting S of Ash. %
I	0·4075	0·2559	6·97	0·7716	51·63	---	---	------	---	----	---	----	---	----	---	----
II	0·2374	0·1497	7·01	0·4526	51·99	---	---	------	---	----	---	----	---	----	---	----
III	0·5898	------	---	------	---	81·3	14·4	767·4	16·61	---	---	----	---	----	---	----
IV	0·2675	------	---	------	---	36·0	14·9	765·5	16·56	----	---	----	---	----	---	----
V	0·6164	------	---	------	---	---	---	------	---	0·0090	1·46	----	---	----	---	----
VI	0·5958	------	---	------	---	---	---	---- -	---	0·0085	1.43	----	---	----	---	----
VII	1·2122	------	---	------	---	---	---	------	---	----	---	0·0048	0·05	----	---	----
VIII	0·5413	------	---	------	---	---	---	------	---	----	---	----	---	0·0483	1·22	1·17
IX	0·4759	------	---	------	---	---	---	------	---	----	---	----	---	0·0403	1·16	1·11

	Percentage composition of the ash-free substance.						Average.
C	52·39	52·74	---	----	----	----	*52·57*
H	7·08	7·11	----	----	----	----	*7·10*
N	----	----	16·85	16·84	----	----	*16·84*
S	----	----	----	----	1·19	1·13	*1·16*
O	----	----	----	----	----	----	*22·33*
							100·00

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ANALYSIS OF MYOSIN I, FROM MUSCLE OF OX.

No.	Substance used gram.	H_2O found. gram.	H %	CO_2 found. gram.	C %	N found. c.c.	N found. T. °C.	N found. Pressure. mm.	N %	Ash found. gram.	Ash. %	$BaSO_4$ from the Ash. gram.	S of Ash. % of sub.	$BaSO_4$ after fusion with $KOH + KNO_3$ gram.	S %	S after deducting S of Ash. %
I	0·3665	0·2313	7·01	0·7053	52·48	---	---	------	---	----	---	----	----	----	---	----
II	0·4900	------	---	0·9404	52·48	---	---	------	---	----	---	---	----	----	---	----
III	0·2913	0·1868	7·13	0·5596	52·38	---	---	------	---	----	---	----	----	----	---	----
IV	0·7746	------	---	------	---	109·1	15·4	759·8	16·73	----	---	----	----	----	---	----
V	0·5013	------	---	------	---	70·6	14·7	757·8	16·72	----	---	----	----	----	---	----
VI	0·7524	------	---	------	---	105·9	15·0	760·7	16·77	----	---	----	----	----	---	----
VII	0·6629	------	---	------	---	---	---	------	---	0·0043	0·65	----	----	----	---	----
VIII	0·6741	------	---	------	---	---	---	------	---	0·0046	0·68	---	----	----	---	----
IX	1·0959	------	---	------	---	---	---	------	---	----	---	0·0020	0·025	----	---	----
X	0·4967	------	---	------	---	---	---	------	---	----	---	----	----	0·0458	1·26	1·23
XI	0·5992	------	---	------	---	---	---	------	---	----	---	----	----	0·0552	1·28	1·25

Percentage composition of the ash-free substance.

									Average.
C	52·82	52·83	52·73	----	----	----	----	----	*52·79*
H	7·06	----	7·17	----	----	----	----	----	*7·12*
N	----	----	----	16·86	16·83	16·88	----	---	*16·86*
S	----	----	----	----	----	----	1·25	1·27	*1·26*
O	----	----	----	----	----	----	----	----	*21·97*
									100·00

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RÉSUMÉ OF THE ANALYTICAL RESULTS.

	C	H	N	S	O
Myosin A, from ox	52·84	7·12	16·89	1·49	21·66
" B, " "	52·51	7·09	16·53	1·25	22·62
" C, " sheep	53·24	7·12	16·45	1·23	21·96
" D^{I}, " calf	52·84	7·11	17·14	1·33	21·58
" D^{II}, " "	52·97	7·13	16·96	1·25	21·69
" E, " halibut	52·39	6·97	16·74	----	----
" F^{I}, " ox	52·99	7·11	16·73	1·29	21·88
" F^{II}, " "	52·88	7·10	16·74	1·31	22·02
" G^{I}, " "	53·05	7·19	16·52	1·18	22·06
" G^{II}, " "	52·82	7·11	16·80	1·16	22·11
" H^{I}, " sheep	52·84	7·10	16·91	1·28	21·87
" H^{II}, " "	52·57	7·10	16·84	1·16	22·33
" I, " ox	52·79	7·12	16·86	1·26	21·97
Average	52·82	7·11	16·77	1·27	21·90
Fibrinogen*	52·93	6·90	16·66	1·25	22·26
Fibrin*	52·68	6·88	16·91	1·10	22·48
Paraglobulin*	52·71	7·01	15·85	1·11	23·32
Egg albumin†	52·18	6·93	15·81	1·87	23·21

* Hammarsten, Jahresbericht der Thierchemie, vol. x, p. 11.

† Chittenden and Bolton. Studies from Laboratory of Physiological Chemistry, Yale University, vol. ii, p. 134.

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The two products are seen to be practically identical in composition, and show close agreement with the other preparations of myosin.

Myosin I, from muscle of ox.

This, the last product analyzed, was obtained from an ammonium chloride extract of the washed muscle tissue from a freshly killed ox, by dialysis. After being washed and dried at 110° C. it was analyzed with the results shown in the accompanying table.

On comparing now the composition of the thirteen preparations of myosin analyzed, there is seen to be a very close agreement throughout. Further, on comparing the average of the analytical results with Hammarsten's figures for fibrinogen, the composition of the two bodies is seen to be almost identical. Compared with egg albumin, the most striking difference in composition is the low content of sulphur, and even if we assume with Danilewsky* that the sulphur of the ash comes from the sulphur of the myosin the correction therefor would not materially lessen the difference. Myosin also contains nearly one per cent. more nitrogen than egg albumin.

Coagulation of myosin solutions.

According to Kühne,† the coagulation of myosin in a 10 per cent. sodium chloride solution occurs at 55–60° C. Weyl‡ obtained similar results. Danilewsky§ states that a 12–15 per cent. ammonium chloride solution of myosin becomes somewhat turbid at 42–43° C., decidedly turbid at 45–50° C., while at 55° C. a flocculent precipitate separates. The same investigator also found that the more concentrated the ammonium chloride solution was, the lower the temperature at which the turbidity and separation of a precipitate occurred, although never below 40° C. In common with other proteid bodies a slight degree of acidity lowers the temperature of coagulation, while a corresponding degree of alkalinity raises it. According to the recent

* Zeitschrift für Physiologische Chemie, Band v, p. 161.

† Lehrbuch der Physiologischen Chemie, p. 275.

‡ Zeitschrift für Physiologische Chemie, Band i, p. 77.

§ Zeitschrift für Physiologische Chemie, Band v, p. 160.

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work of Halliburton,* muscle-clot or myosin is formed from a substance (myosinogen) in the muscle-plasma which coagulates by heat at 47° C. and 56° C., thus indicating that it probably consists of two distinct proteids which coagulate at these temperatures respectively. Further, Halliburton considers that whenever myosin is dissolved in a suitable saline solution, it is at once re-converted into myosinogen, or rather into two proteids which resemble myosinogen in that they have the same heat coagulation temperatures and that they are convertible into myosin by dilution of their saline solutions. In salted muscle-plasma, Halliburton recognizes five distinct proteid bodies, distinguishable by fractional heat coagulation, viz :

47° C., a flocculent, somewhat sticky precipitate.
56° C., a more abundant and very sticky precipitate.
63° C., a finely flocculent precipitate, not sticky.
73° C., a finely flocculent precipitate, not sticky.
A non-coagulable albumose.

Of these, the two proteids coagulating at 47° C. and 56° C., make up the muscle-clot or myosin.

With these preliminary statements, we proceed to the results obtained in our study of the coagulation points of the different myosins, simply prefacing it with the explanation that our experiments were performed previous to reading Halliburton's paper. Naturally, the temperature of coagulation offers the simplest and surest means, in conjunction with the determination of composition, of detecting any difference in the character of the myosins from different sources. And with this as the main object the following experiments were tried. In every experiment, the muscle tissue was chopped quite fine and very thoroughly extracted with water, well thymolized, prior to solution of the myosin with the saline fluid.

We first demonstrated to our satisfaction that a 5 per cent. ammonium chloride solution of myosin invariably coagulates at a lower temperature than a 5 per cent. sodium chloride solution, and further, that the original saline extract of washed muscle shows approximately the same temperature of coagulation as the salt solution of precipitated myosin.

* Journal of Physiology, vol. viii, p. 148.

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		Turbidity.	Flocculent precipitate.
A.	5 per cent. NH_4Cl extract of sheep's muscle.	43° C.	47° C.
B.	5 per cent. NH_4Cl solution of H_2O precipitate of myosin, from A.	44°	48°
C.	5 per cent. NaCl solution of H_2O precipitate of myosin, from A.	57°	62°
D.	5 per cent. NH_4Cl extract of ox muscle.	40°	44°
E.	5 per cent. NaCl solution of H_2O precipitate of myosin, from D.	57°	62°
F.	5 per cent. NH_4Cl extract of lamb's muscle.	44°	47°
G.	5 per cent. NaCl solution of H_2O precipitate of myosin, from F.	51°	57°
H.	5 per cent. NH_4Cl extract of calf's muscle.	41°	44°
I.	5 per cent. NaCl extract of same.	53°	56°
J.	5 per cent. NH_4Cl extract of breast muscle from chicken.	43°	46°
K.	5 per cent. NaCl extract of same.	53°	57°
L.	5 per cent. NH_4Cl extract of leg muscle from chicken.	45°	48°
M.	5 per cent. NaCl extract of same.	56°	61°

In all of these trials, the filtrate from the flocculent precipitate failed to show any further coagulation, although the temperature was raised to above 75° C.

The results show a varying difference in the coagulation points of the ammonium and sodium chloride solutions, but on an average the difference amounts to eleven degrees. It further appears that the myosin solutions (in NaCl) from lamb, calf, and chicken muscle have a somewhat lower coagulating point than the corresponding extracts from ox and sheep muscle.

In another series of results, shown in the accompanying table, the same difference in coagulation point shows itself, and it is further seen that the extracts from rabbit's and halibut's muscle have a still lower coagulating point. Further, in this series of experiments, the filtrate from the first coagulum gave a second precipitate on raising the temperature, and in the case of the muscle from ox and lamb, a third coagulum was obtained at 74° C.

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COAGULATION OF MYOSIN SOLUTIONS.

Nature of the Solution.	Coagulation of the Solution.		Coagulation of the filtrate.	
5 per cent. NaCl.	Turbidity.	Flocculent precipitate.	Turbidity.	Flocculent precipitate.
Sheep's muscle.	57° C.	63° C.	68° C.	73° C.
Ox "	58°	63°	66°	72°
Rabbit's "	52°	57°	62°	66°
Pig's "	56°	60°	65°	70°
Lamb's "	53°	60°		
Calf's "	53°	57°	66°	70°
Halibut's "	47°	53°	55°	60°
Ox "	57°	62°	64°	66°
Lamb's "	55°	62°	63°	68°
Calf's "	53°	59°	63°	66°
Chicken " (breast)	53°	57°	61°	67°
" " (leg)	56°	61°		
Lobster* "	65°			

The results collectively fail to show the presence of a proteid coagulating at 47° C., given by Halliburton as characteristic of myosin, but they do show a coagulum at 56° C. or thereabouts, corresponding to Halliburton's second proteid. This difference is possibly due to the character of the saline solution, Halliburton having used a magnesium or ammonium sulphate solution. The coagulums obtained at higher temperatures show an approach, at least, to the figures found by Halliburton in his fractional coagulation of salted muscle-plasma.

*Lobster did not yield enough myosin to permit of an exact determination, still a decided turbidity was present at 65° C. The addition of water to the 5 per cent. $NaCl$ solution, gave a flocculent precipitate which changed to a glairy mass resembling much the white of an egg. On washing with water it became insoluble, but differed in general appearance from similar precipitates from other sources.

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A careful study of the preceding results, combined with what has been known concerning the chemical properties of myosin, seems to justify the assumption that myosin, as it occurs throughout the animal kingdom, is a single chemical compound, doubtless formed, as suggested by Halliburton, by the interaction of one or more myosinogens and a ferment body. That myosin is a single body, is supported by the observed agreement in chemical composition and the general uniformity in the temperature of coagulation of myosin from various animal sources, and is furthermore to be inferred from the similarity in function of the tissue in which it occurs most abundantly.

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Myosinoses. By W. Kühne and R. H. Chittenden.

Of the primary digestion products of the various proteids, the albumoses from fibrin * and egg albumin † have been more or less carefully examined and analyzed, as also the globuloses,‡ the caseoses,§ and elastinoses. ‖ Further, the vitelloses ¶ have likewise been prepared from crystalline phyto-vitellin and their properties ascertained. With the hope of gradually completing the list we have undertaken a study of the primary digestion products of myosin, the results of which we now present.

For the preparation of myosin, large quantities of finely divided ox muscle were extracted with cold water until the fluid no longer gave reaction for albumin, after which the tissue was placed in an excess of a 15 per cent. ammonium chloride solution, and the myosin ultimately precipitated from the filtered fluid by dialysis.** The extraction of myosin with ammonium chloride, after the method of Danilewsky,†† is far better in every way than the older method of extraction with 10 per cent. sodium chloride, since myosin is dissolved more rapidly and completely by the ammonium salt, even from coarsely divided muscle, and at the same time forms a more easily filterable fluid. Further, the advantage of precipitating the myosin by dialysis in running water, instead of pouring the ammonium chloride solution into a large excess of water, consists in the avoidance of the large volumes of fluid necessary in the preparation of such a large quantity of myosin, while at the same time the myosin is obtained equally free from soluble salts. At the best, however, the preparation of such a quantity of myosin as was needed in the present investigation involved a large amount of labor and a comparatively low temperature, hence compelling us to take the winter season for the work. The united products obtained in this manner were treated ultimately with dilute alcohol, whereby the semi-gelatinous mass was naturally more or less shrunken, and finally with ether.

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* Zeitschrift für Biologie, Band xix, p. 159 and Band xx, p. 11.

† Studies from Laboratory of Physiological Chemistry, Yale University, vol. ii, p. 126.

‡ Zeitschrift für Biologie, Band xxii, p. 409.

§ This volume, p. 66. ‖ This volume, p. 19.

¶ Ueber vitellosen, by Dr. R. Neumeister. Zeitschrift für Biologie. Band xxiii, p. 2.

** See the preceding article.

†† Zeitschrift für Physiologische Chemie, Band v, p. 158

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A sample of the product, carefully dried at 110° C. until of constant weight, yielded the following results on analysis.

Myosin.

I. 0·3665 gram substance gave 0·2313 gram H_2O = 7·01 per cent. H and 0·7053 gram CO_2 = 52·48 per cent. C.

II. 0·4900 gram substance gave 0·9404 gram CO_2 = 52·48 per cent. C.

III. 0·2913 gram substance gave 0·1868 gram H_2O = 7·13 per cent. H and 0·5596 gram CO_2 = 52·38 per cent. C.

IV. 0·7746 gram substance gave 109·1 c.c. N at 15·4° C. and 759·8 mm pressure = 16·73 per cent. N.

V. 0·5013 gram substance gave 70·6 c.c. N at 14·7° C. and 757·8 mm pressure = 16·72 per cent. N.

VI. 0·7524 gram substance gave 105·9 c.c. N. at 15·0° C. and 760·7 mm pressure = 16·77 per cent. N.

VII. 0·6629 gram substance gave 0·0043 gram ash = 0·65 per cent.

VIII. 0·6741 gram substance gave 0·0046 gram ash = 0·68 per cent.

IX. The ash from 1·3370 grams substance gave 0·002 gram $BaSO_4$ = 0·02 per cent. S.

X. 0·4967 gram substance gave by fusion with KOH + KNO_3, after Hammarsten's method, 0·0458 gram $BaSO_4$ = 1·26 per cent. S; deducting 0·02 per cent. S of ash = 1·24 per cent.

XI. 0·5992 gram substance gave after fusion with KOH + KNO_3 0·0552 gram $BaSO_4$ = 1·28 per cent. S; deducting 0·02 per cent. = 1·26 per cent.

Percentage composition of ash-free myosin.

									Average.
C	52·82	52·83	52·73	----	----	----	----	----	52·79
H	7·06	----	7·17	----	----	----	----	----	7·12
N	----	----	----	16·86	16·83	16·88	----	----	16·86
S	----	----	----	----	----	----	1·25	1·27	1·26
O	----	----	----	----	----	----	----	----	21·97
									100·00

The ash consisted almost entirely of calcium phosphate.

Digestion of Myosin.

The myosin purified as described with alcohol and ether, proved so resistant to the action of pepsin-hydrochloric acid, that its diges-

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tion could be accomplished only by repeated treatment with the ferment. The gastric juice at first emyloyed was prepared by warming 120 grams of mucous membrane from a pig's stomach with 1200 c. c. 0·4 per cent. hydrochloric acid for 24 hours at 41° C., filtration through paper, dilution of the acid fluid with an equal volume of water and further exposure to a temperature of 40° C. for four days, in order to convert any dissolved albumoses into peptone.

The digestive fluid so obtained contained 0·2 per cent. HCl and 0·5 per cent. solid matter.

200 grams of finely powdered myosin were placed in 2 litres of this gastric juice, 2 litres of 0·2 per cent. hydrochloric acid added and the whole warmed at 40° C. for two days. As only a little of the myosin appeared to be dissolved, the acidity was increased to 0·4 per cent. and the mixture continued at 40° C. for 24 hours longer. Although a large residue still remained undissolved, the entire mixture was made neutral with sodium hydroxide and strained through a cloth filter. The undigested residue, together with the abundant neutralization precipitate, was again warmed for several days at 40° C. with 2 litres of 0·4 per cent. hydrochloric acid containing 7·5 grams of scrapings from a stomach mucous membrane. After stopping the action of the pepsin by neutralization, this second digestive fluid was added to the first. In spite of the energetic action of the pepsin (tested by allowing a little of the solution to act on boiled fibrin), there still remained considerable undissolved substance together with considerable neutralization precipitate, the whole apparently very resistant to the action of the ferment. In the united solutions there was present less than 100 grams of organic matter, of which it is fair to presume about 10·5 grams consisted of impurity in the form of substances from the stomach membrane. When it is remembered, however, that well prepared gastric juice contains only a very small amount of substances precipitable by the salts used in separation of the proteoses, it is fair to assume that this impurity in the digestive fluid is unimportant in the study of the myosinoses. In all, nearly 60 grams of myosinoses were obtained.

For separation of the myosinoses, the united neutral filtrates were concentrated to about one-sixth of their volume and saturated with crystals of rock salt, by which the fluid was converted into a gelatinous mass. On adding saturated salt solution to a portion of the filtered fluid it was rendered decidedly turbid; consequently, three volumes of a saturated sodium chloride solution were added to the mixture, after which it was found that neither salt in substance or in solution

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would give any further turbidity. From this it is evident that the neutral digestive fluid is not completely precipitated by simple saturation with sodium chloride, but that complete precipitation is reached only when the absolute quantity of salt stands in a certain proportion to the proteoses present.

After this separation of the first portion of the myosinoses, which would naturally consist of proto, hetero, and dysmyosinose, the remainder were precipitated first with salt-saturated 30 per cent. acetic acid and then, after removal of the sodium chloride by dialysis, with neutral ammonium sulphate. In the solution remaining from this last precipitation, peptone was detected by the biuret reaction.

Protomyosinose.

The sodium chloride precipitate, after thorough washing with saturated salt solution, dissolved almost entirely on being rubbed up with water.

What did remain undissolved, showed the reactions of dysalbumose. It was insoluble in water and in salt solutions of all strengths, but easily soluble in 0·1 per cent. hydrochloric acid, much more difficultly soluble in 0·5 per cent. sodium carbonate, by neutralization only partially precipitated and gave the biuret reaction. From the filtrate of the neutralized hydrochloric acid solution, some heteromyosinose in the form of a flocculent precipitate was obtained by dialysis. This hetero body was soluble in dilute sodium chloride solutions, insoluble in water, precipitable by salt in substance, and after the manner of the albumoses gave a precipitate with nitric acid in the cold, which disappeared as the mixture was warmed, reappearing as the solution cooled. This heteromyosinose, formed from dysmyosinose by a process of retrogression, amounted to considerable; about 1·5 grams.

Reactions of Protomyosinose.

In order to purify protomyosinose the aqueous solution of the substance was freed from sodium chloride by dialysis, by which only traces of heteromyosinose separated, and the solution evaporated to a thin syrup. The fluid was filtered from a slight flocculent albumin-like precipitate insoluble in hot water, somewhat further concentrated and the pure myosinose separated by alcohol. After washing with alcohol and ether it appeared as a light, white powder. The yield amounted to about ten grams.

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The reactions of protomyosinose agree in general with those of protoalbumose, but with one important difference, viz: that an aqueous solution of the former, free from salt, of whatever concentration, is not rendered turbid by nitric acid. Addition of even a little sodium chloride to the acid fluid, however, is sufficient to cause a heavy precipitate, soluble as the mixture is warmed but reappearing as the solution cools. The precipitate is also soluble in an excess of the acid in the cold.

Protomyosinose is readily soluble in distilled water, the solution showing a weak, but unquestionably alkaline reaction. In this solution, as well as in a solution rendered acid by acetic acid, cupric sulphate produces a heavy turbidity, which on boiling almost entirely disappears. Acetic acid and potassium ferrocyanide produce a heavy precipitate, insoluble in glacial acetic acid. Neutral lead acetate gives no precipitate. Basic lead acetate and mercuric chloride both produce a heavy turbidity. An aqueous solution of the myosinose boiled with sodium hydroxide and lead acetate is colored deep brown or black; with sodium hydroxide and cupric sulphate a beautiful red. Concentrated sodium hydroxide, as in protoelastose, produces a heavy flocculent, gelatinous precipitate. By saturation of an aqueous solution of protomyosinose with sodium chloride, only a portion of the substance is precipitated; the portion remaining dissolved is precipitable by acid.

The composition of the substance, dried at 110° C. until of constant weight, is shown by the following analysis.

Protomyosinose.

I. 0·3722 gram substance gave 0·2387 gram H_2O = 7·12 per cent. H and 0·7090 gram CO_2 = 51·95 per cent. C.

II. 0·4239 gram substance gave 0·2688 gram H_2O = 7·05 per cent. H and 0·8048 gram CO_2 = 51·77 per cent. C.

III. 0·3319 gram substance gave 0·2138 gram H_2O = 7·15 per cent. H and 0·6308 gram CO_2 = 51·83 per cent. C.

IV. 0·4798 gram substance gave 67·5 c. c. N at 14·8° C. and 762·1 mm pressure = 16·79 per cent. N.

V. 0·5663 gram substance gave 78·3 c. c. N at 15·1° C. and 768·2 mm pressure = 16·64 per cent. N.

VI. 0·4429 gram substance gave 62·7 c. c. N at 15·8° C. and 758·2 mm pressure = 16·77 per cent. N.

VII. 0·5494 gram substance gave 0·0062 gram ash = 1·13 per cent.

VIII. 0·7080 gram substance gave 0·0081 gram ash = 1·14 per cent.

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IX. The ash from 1·2574 grams substance gave 0·0132 gram $BaSO_4$ = 0·14 per cent. S.

X. 0·4183 gram substance gave after fusion with KOH + KNO_3 0·0450 gram $BaSO_4$ = 1·47 per cent. S; after deducting 0·14 per cent. S of the ash = 1·33 per cent.

XI. 0·3963 gram substance gave after fusion with KOH + KNO_3 0·0411 gram $BaSO_4$ = 1·42 per cent. S; deducting 0·14 per cent. = 1·28 per cent.

Percentage composition of ash-free protomyosinose.

									Average.
C	52·53	52·35	52·41	....	....	....	....	....	52·43
H	7·19	7·12	7·22	....	....	....	....	....	7·17
N	....	....	....	16·98	16·83	16·96	....	....	16·92
S	....	....	....	....	....	....	1·35	1·30	1·32
O	....	....	....	....	....	....	....	....	22·16
									100·00

The ash contained only calcium phosphate and sulphate, with a little ferric oxide.

Deuteromyosinose.

This myosinose, as we have before mentioned, was obtained mainly by saturation with ammonium sulphate. After removal of the greater portion of protomyosinose by saturation with salt as described, deuteromyosinose was in part precipitated by salt-saturated acetic acid, but this precipitate could not be used on account of the large amount of proto body precipitated with it, as shown by the cupric sulphate reaction. This method of treatment, however, although necessitating the loss of considerable deuteromyosinose, enabled us to remove the proto body completely and thus ensure a pure specimen of deuteromyosinose on treatment of the filtrate with ammonium sulphate. Naturally, before saturating the fluid with the ammonium salt, the sodium chloride was removed by dialysis. The myosinose thus precipitated by saturation with ammonium sulphate was dissolved in water and dialyzed until the sulphate was entirely removed, or to such an extent that the fluid gave only the slightest turbidity with barium chloride even on long standing. We hastened the removal of the sulphate by repeated evaporation and renewed dialysis of the concentrated fluid. The substance was finally precipitated from the suitably concentrated fluid, as a white powder, by alcohol and washed with alcohol and ether. It weighed forty-five grams.

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Reactions of deuteromyosinose.

Like the preceding myosinose, this body also reacts alkaline in an aqueous solution and this property exercises more or less of an influence on certain of its reactions. In order to convince ourselves of the absence of traces of either proto or heteromyosinose, we paid particular attention to the behavior of the deutero body towards the cupric sulphate reaction, which according to Neumeister's observations is a decisive test on this point. A fairly concentrated solution of our preparation was not rendered turbid by cupric sulphate in the cold, but after boiling and then cooling the mixture, a slight turbidity appeared. Solutions of the substance so concentrated as to be almost syrupy, gave a slight turbidity at once, the turbidity disappearing when the solution was heated and reappearing as the fluid cooled. By partial neutralization of the alkalinity of the myosinose solution, leaving the fluid, however, still alkaline to delicate test papers, precipitation by cupric sulphate was entirely prevented. As protomyosinose is precipitated by the copper salt equally well in an acid fluid, we are led to consider our deuteromyosinose entirely free from this impurity.

In general, deuteromyosinose shows much the same reactions as deuteroalbumose, but is somewhat different from the latter in that it is more difficultly precipitable. Acetic acid and nitric acid produce a precipitate only after addition of sodium chloride to saturation. Acetic acid and potassium ferrocyanide give a decided turbidity, not soluble in glacial aectic acid. Basic lead acetate and mercuric chloride both produce a precipitate, insoluble in excess of the reagent. Cold nitric acid quickly produces an intense yellow color. The biuret reaction comes out distinctly, but on boiling the myosinose with sodium hydroxide and lead acetate only a faint browning of the fluid is obtained.

The composition of the substance, dried at 110° C., is shown by the following analysis:

Deuteromyosinose.

I. 0·2121 gram substance gave 0·1394 gram H_2O = 7·30 per cent. H and 0·3896 gram CO_2 = 50·09 per cent. C.

II. 0·2115 gram substance gave 0·1390 gram H_2O = 7·30 per cent. H and 0·3891 gram CO_2 = 50·12 per cent. C.

III. 0·3830 gram substance gave 53·6 c. c. N at 15·0° C. and 762·5 mm pressure = 16·71 per cent. N.

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IV. 0·2940 gram substance gave 41·4 c. c. N at 15·7° C. and 759·7 mm pressure = 16·72 per cent. N.

V. 0·4572 gram substance gave 0·0080 gram ash = 1·77 per cent.

VI. 0·5462 gram substance gave 0·0094 gram ash = 1·72 per cent.

VII. The ash from 1·0034 grams substance gave 0·0116 gram $BaSO_4$ = 0·15 per cent. S.

VIII. 0·3343 gram substance gave after fusion with KOH + KNO_3 0·0315 gram $BaSO_4$ = 1·29 per cent. S; after deducting 0·15 per cent. S of the ash = 1·14 per cent.

IX. 0·4050 gram substance gave after fusion with KOH + KNO_3 0·0420 gram $BaSO_4$ = 1·42 per cent. S; deducting 0·15 per cent. = 1·27 per cent.

Percentage composition of ash-free deuteromyosinose.

							Average.
C	50·95	50·98	....	....	....	....	50·97
H	7·42	7·42	....	....	....	....	7·42
N	....	....	17·00	17·01	....	....	17·00
S	....	....	....	....	1·16	1·28	1·22
O	....	....	....	....	....	....	23·39
							100·00

The ash consisted only of calcium phosphate and sulphate, with some oxide of iron.

This is as far as we have been able at present to carry our study of the myosinoses, since hetero and dysmyosinose appear to have been present in the digestions only in very small quantity. Myosin purified by alcohol, as was the preparation employed by us, is so difficultly digestible that it is attacked only by the most energetic pepsin mixture, and this has the disadvantage of rapidly converting hetero and protoproteose, which according to Neumeister's * investigations are formed in the beginning of digestion, into the deutero body; consequently in the present instance we could expect a large amount only of deuteromyosinose. Corresponding with this view, we obtained for 45 grams of deuteromyosinose, only 10 grams of protomyosinose and but 3 grams of hetero and dysmyosinose. Further, the unavoidable loss attending the separation of these bodies was probably greater with deuteromyosinose than with the others. Portions of the fluid from the first and second digestion, tested before they were united, showed also a difference in that the fluid from the first digestion con-

* Loc. cit.

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tained far more proto and dysmyosinose than the latter, which, on the other hand, was particularly rich in deuteromyosinose. From this it is evident how an albuminous body can in one sense be difficultly digestible, in that its solution takes place slowly and its primary cleavage products form gradually, and yet the latter be further transformed, under the continued action of the ferment, far more readily and completely. Our conception of digestibility needs therefore to be broadened, after having for so long embraced simply the time required for solution of the proteid, or, in the case of pepsin digestion, the hardly attainable extreme of complete conversion into peptone.

On comparing now the results of the analyses of myosin and the two myosinoses in the following table,

	C	H	N	S	O
Myosin	52·79	7·12	16·86	1·26	21·97
Protomyosinose	52·43	7·17	16·92	1·32	22·16
Deuteromyosinose	50·79	7·42	17·00	1·22	23·39

there is seen to be only a small difference in composition between myosin and protomyosinose. The content of carbon in the latter is only 0·36 per cent. less than in the former, that of nitrogen 0·06 per cent. greater and oxygen only 0·17 per cent. greater. Between deuteromyosinose and the undigested proteid, on the other hand, there is a far greater difference in composition, the content of carbon being 1·82 per cent. less, while nitrogen is 0·14 per cent. greater and oxygen 1·42 per cent. greater. In hydrogen, both myosinoses show a small increase over myosin, while the content of sulphur is practically unaltered.

That portion of the myosin, which was apparently not further alterable by gastric juice, together with the somewhat large neutralization precipitate, we attempted to digest by the action of trypsin. This was only partially successful, for although the trypsin solution consisted of an extract from 20 grams of dry pancreas in 2 litres of 0·4 per cent. sodium carbonate, and the proteid matter was warmed with it at 40° C. for six days, we were not able to bring more than half of the material into solution. The digestive fluid behaved somewhat peculiarly, in that with a certain excess of acetic acid it gave a fine pulverulent white precipitate. After removal of this substance, the solution gave no turbidity whatever with sodium chloride, or with sodium chloride and nitric acid, and only a very slight one with ammonium sulphate ; hence it contained no myosinoses. Peptone, however, was formed in considerable quantity.

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The Relative Absorption of Nickel and Cobalt. By R. H. Chittenden and Charles Norris, Jr., Ph.B.

When nickel and cobalt were first discovered they were supposed to be possessed of decided toxic properties, and nickel particularly was looked on as more poisonous than copper. Examination, however, of many of the supposed cases of nickel poisoning led to the view that toxic action was due, mainly at least, to the presence of arsenical impurities, with which German nickel particularly was known to be contaminated. Gradually, therefore, the view has become widespread that nickel and cobalt are no more poisonous than iron, with which chemically they are so closely related. We have not, however, been able to find many very definite statements regarding their physiological or toxic action. Blake * in his study of the relation between isomorphism, molecular weight, and physiological action, places the sulphates of nickel and cobalt in the same group with copper, zinc, iron, etc., and further arranges cobalt, copper and zinc together in a sub-group, on account of their arresting the action of the heart and preventing the coagulation of the blood. Nickel, however, is placed with manganese on account of its exerting a marked influence on the nervous system. As to the intensity of their physiological action, Blake apparently considers nickel and copper of the same strength, while cobalt is figured as one-twentieth stronger. Both salts, however, kill by arresting the action of the heart and in lethal action cobalt stands first.† Woodman and Tidy ‡ state that 30 grains of the oxide of cobalt given to a dog proved fatal in a few hours, whilst 3 grains of the sulphate injected into a vein proved fatal in four days. With nickel, the same writers state that vomiting is freely induced in a dog by a dose of 20 grains of the sulphate, whilst 10 grains injected into the jugular vein will destroy life instantly. Finally, Brunton and Cash have found that nickel and cobalt, like most other metallic salts, cause slight contraction of the blood vessels.§

* American Journal of Science and Arts, vol. vii, p. 194.

† See Brunton's Pharmacology and Therapeutics, p. 51.

‡ Forensic Medicine and Toxicology, p. 171 and p. 214.

§ Brunton's Pharmacology, p. 246.

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Our experiments have been conducted wholly upon rabbits, the main object being to study the distribution of the absorbed poison. The salts used were chemically pure cobalt and nickel nitrates, dried over sulphuric acid. They were administered by mouth in gelatin capsules.

We first endeavored to gain some idea of the relative toxic action of the two salts. For this purpose two rabbits, weighing 2 kilos. each, were dosed as follows :

Experiment I.

		Rabbit A.		Rabbit B.	
Oct. 1,	10 a.m.	0·150 gram	$Ni(NO_3)_2$	0·150 gram	$CO(NO_3)_2$
" 1,	4 p.m.	0·102 "	"	0·100 "	"
" 2,	10 a.m.	0·150 "	"	0·150 "	"
" 2,	5 p.m.	0·101 "	"	0·100 "	"
" 3,	9 a.m.	0·200 "	"	0·200 "	"
" 3,	4 p.m.	0·200 "	"	0·200 "	"
" 4,	9 a.m.	0·251 "	"	0·250 "	"
" 4,	5 p.m.	0·250 "	"	0·250 "	"
" 5,	10 a.m.	0·350 "	"	0·350 "	"
" 5,	4 p.m.	0·350 "	"	0·350 "	"
" 6,	9 a.m.	0·500 "	"	0·500 "	"
" 6,	3 p.m.	0·500 "	"	0·500 "	"
		3·104 grams.		3·100 grams.	

Both rabbits died on the morning of the 7th, apparently from heart failure. The urine was examined each day, but in both cases was entirely free from either sugar or albumin. On the 3d, the cobalt rabbit appeared troubled with involuntary micturition and defecation, and on the 5th there was quite pronounced partial paralysis of the hind quarters. The appetite remained good up to the 6th. On the 6th instant, after administration of 500 milligrams of the cobalt salt, there was a loose diarrhœa continuous till death, decided paralysis of the hind legs, total loss of appetite with a decided lowering of the body temperature. After death, the body weight was found to have diminished three-fifths of a kilo. With the nickel rabbit, there was no diarrhœa whatever and the paralysis of the hind legs, visible on the 6th, was not as pronounced as with cobalt. The loss of body weight was the same as in the cobalt rabbit.

On post-mortem, the only noticeable abnormal feature with either cobalt or nickel was a slight congestion of the lining membrane of the stomach and intestines. The stomach, however, was found full of undigested food, as if the salt had interfered with the digestive process.

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It is thus seen that neither the cobalt or nickel salt can be called a violent poison, since comparatively large amounts are required to produce a toxic effect, and even then the action is somewhat slow. This is still more clearly seen in the next experiment.

Experiment II, with cobalt.

A vigorous black doe, weighing 2 kilos., was dosed as follows :

Oct.	8,	0·200	gram	cobalt	nitrate.
"	9,	0·400	"	"	"
"	10,	0·600	"	"	"
"	11,	0·700	"	"	"
"	12,	0·250	"	"	"
"	13,	0·250	"	"	"
"	14,	0·250	"	"	"
"	15,	0·550	"	"	"
"	16,	1·000	"	"	"
"	17,	1·500	"	"	"
"	18,	2·000	"	"	"
		7·700 grams.			

The animal died on the 19th, of heart failure. On the 11th, when 7 decigrams of the salt were given, the animal appeared sickly, with loss of appetite, high rectal temperature, etc., but by diminishing the dose of cobalt the animal rapidly recovered. On the 17th, however, with increase in the dose of salt there was diarrhœa, with a slight indication of paralysis of the extremities. At no time did the urine contain either sugar or albumin. There was a decided loss of body weight, nearly one-third. On post-mortem, the stomach and small intestines were found somewhat inflamed, and the liver showed signs of a slight fatty degeneration.

Immediately on the death of the animal the internal organs were removed and the absorbed cobalt determined.

The method of analysis, both for cobalt and nickel, was as follows: The finely divided tissue was oxidized with dilute hydrochloric acid and potassium chlorate, after the usual method. From the solution so obtained, chlorine was removed by evaporation, the fluid made alkaline with ammonia and the cobalt or nickel precipitated by a stream of hydrogen sulphide gas. The washed sulphide, after ignition, was then dissolved in nitro-hydrochloric acid, the free acid entirely removed by heat and the chloride converted into sulphate by addition of concentrated sulphuric acid. Ultimately, the sulphuric acid solution of sulphate was diluted somewhat with water, made strongly alkaline with ammonia and the metal separated by electrolysis.

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Following are the amounts of absorbed cobalt found in experiment II:

	Total weight of organ.	Weight of Co.	Co per 100 grams of tissue.
	grams.	milligrams.	milligrams.
Stomach and contents	81·75	57·5	70·34
Small intestines	43·90	13·8	31·44
Large intestines	26·40	40·2	152·10
Cæcum	154·00	302·5	196·43
Liver	45·75	8·9	19·43
Kidneys	10·30	lost	----
Heart	8·80	0·7	7·95
Lungs	5·82	0·9	15·46
Muscle of legs	100·00	1·9	1·90
Muscle of back	27·45	1·5	5·46
Brain	8·75	0·8	6·45
Spinal cord	3·63		

Considering the large amount of cobalt nitrate administered, the extreme solubility of the salt, and the length of time intervening between the first and last dose, it is somewhat surprising that the amount absorbed was not greater. Evidently a large portion of the cobalt passes directly through the alimentary canal, probably combining with the proteid matter of the food to form an insoluble and indigestible compound.

Experiment III, with nickel.

A black and white doe of 4 kilos. body weight was dosed as follows:

Oct. 15,	0·200	gram	nickel	nitrate.
" 16,	0·400	"	"	"
" 17,	0·600	"	"	"
" 18,	0·800	"	"	"
" 19,	0·500	"	"	"
	2·500 grams.			

On the morning of the 20th, the animal was found dead, the only noticeable symptoms having been general weakness, loss of appetite and diarrhœa. The loss of body weight was quite pronounced, amounting in the six days to 1·4 kilos. The urine was entirely free from albumin and sugar. There was a little inflammation of the stomach. In this experiment, the toxic action would appear to have been greater than that of the cobalt in the preceding experiment. The distribution of the absorbed poison was as follows:

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	Total weight of organ. grams.	Weight of Ni. milligrams.	Ni per 100 grams of tissue. milligrams.
Stomach and contents	115·00	12·2	10·68
Small intestines	67·87	11·4	16·88
Large intestines	37·45	3·9	10·41
Cæcum	190·00	9·4	4·95
Liver	87·50	5·1	5·83
Kidneys	18·85	0·8	4·24
Heart	8·90	1·3	14·60
Lungs	14·15	0·9	6·36
Muscle of legs	292·00	1·2	0·41
Muscle of back	129·00	3·8	2·95
Brain	8·75	2·1	24·00
Spinal cord	5·00	1·0	20·00
Spleen	2·00	0·6	30·00

The amount of nickel found in the alimentary tract is naturally not so large as in the case of cobalt, where the final doses were larger and the diarrhœa not so bad. Of the absorbed nickel, the distribution is essentially the same as with cobalt. The amount in the kidneys and liver is not as large as would be expected from the size of the doses and the soluble character of the salt. It suggests that only a small portion of the salt given is absorbed, and that elimination goes on with comparative slowness. Quite striking is the peculiar distribution of the nickel in the muscle tissue, the amount in the muscles from the back being seven times as large as in the leg muscles. The same peculiarity is likewise noticeable with cobalt. Also noticeable is the comparatively large amount in the brain and spinal cord, more of the poison in proportion to the weight of the organ being found here than in either the liver or kidneys.

Experiment IV, with cobalt and nickel.

In this experiment, two rabbits of approximately the same body weight were dosed with nickel and cobalt respectively as follows :

	Rabbit A.	*Rabbit B.*
Nov. 1,	0·200 gram $CO(NO_3)_2$.	0·200 gram $Ni(NO_3)_2$.
" 2,	0·150 " "	0·350 " "
" 3,	0·250 " "	0·200 " "
" 4,	0·400 " "	0·300 " "
" 5,	0·250 " "	0·500 " "
" 6,	0·800 " "	0·300 " "
" 7,	0·500 " "	0·800 " "
" 8,	0 " "	0·500 " "
	2·550 grams.	3·150 grams.

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Both animals were found dead on the 9th. In the cobalt rabbit, the stomach, lungs, kidneys and brain were found more or less congested and there was considerable diarrhœa before death. In the nickel rabbit, there was no diarrhœa and but little congestion. Rectum was found full of hard fæces.

Following is the distribution of the poison in the two rabbits.

Rabbit A, cobalt.

	Total weight of organ. grams.	Weight of Co. milligrams.	Co per 100 grams of tissue. milligrams.
Stomach and contents	76·65	10·8	14·09
Small intestines	58·70	7·5	12·77
Large intestines	20·10	10·9	54·23
Cæcum	108·00	50·5	46·76
Liver	91·08	3·3	3·62
Kidneys	13·55	0·8	5·90
Heart	13·37	0·9	6·73
Lungs	9·62	1·3	13·50
Muscle of legs	196·00	1·2	0·61
Muscle of back	95·85	1·7	1·77
Brain	8·87	1·2	13·53
Spinal cord	4·95	0·9	18·18

Rabbit B, nickel.

	Total weight of organ. grams.	Weight of Ni. milligrams.	Ni per 100 grams of tissue. milligrams.
Stomach and contents	64·80	18·4	28·39
Small intestines	52·30	2·0	3·82
Large intestines	12·10	2·0	16·53
Liver	70·00	5·6	8·00
Kidneys	11·20	0·8	7·05
Heart	6·72	0·8	11·90
Lungs	7·92	2·1	26·51
Muscle of leg	157·00	1·1	0·70
Muscle of back	82·00	2·0	2·44
Brain	9·00	1·2	13·33
Spinal cord	3·98	1·7	42·71

Very noticeable in both of these results, as in the preceding experiments, is the comparatively large amount of poison in the brain and spinal cord, also the same relative distribution in the muscle tissue of the legs and back previously commented upon. The large amount of poison in the lungs and heart, as contrasted with the liver and kidneys, is also quite noticeable.

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Experiment V, with nickel.

In this experiment, 1·8 grams of nickel nitrate were given by mouth during five days to a rabbit of 1·5 kilos. weight, the individual doses being of about the same size as in preceding experiments. On the sixth day, the animal died of heart failure. Following is the distribution of the poison.

	Total weight of organ. grams.	Weight of Ni. milligrams.	Ni per 100 grams of tissue. milligrams.
Stomach and contents	73·00	3·2	4·38
Small intestines	59·10	1·8	3·04
Large intestines	36·40	9·9	27·20
Cæcum	176·00	36·5+	20·74+
Liver	58·00	8·1	13·96
Kidneys	10·27	0·8	7·79
Heart	7·42	1·3	16·63
Lungs	6·52	0·2	3·06
Muscle of legs	97·30	2·6	2·67
Muscle of back	35·00	3·7	10·57

Experiment VI, with cobalt.

In this experiment, an attempt was made to ascertain something regarding the elimination of the cobalt through the kidneys. For this purpose, the urine was collected each day and the cobalt determined by the same method as used in the analysis of the organs. The rabbit (body weight 2·5 kilos.) was dosed as follows:

		Examination of the 24 hours' urine.
Nov. 27,	0·100 gram $CO(NO_3)_2$.	30 c.c. contained 1·3 milligrams Co.
" 28,	0·200 " "	50 " " 6·1 " "
" 29,	0 " "	73 " " 6·2 " "
" 30,	0·300 " "	
Dec. 1,	0·200 " "	
" 2,	0 " "	120 " " 23·3 " "
" 3,	0·400 " "	
" 4,	0·400 " "	
" 5,	0·400 " "	
" 6,	0·400 " "	
" 7,	0·400 " "	
" 8,	0·500 " "	
" 9,	0 " "	
" 10,	0·500 " "	
" 11,	0·500 " "	
	4·300 grams.	

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The animal died on the 12th. On the last day, 15 c. c. of thick, dark brown, viscid urine were voided which contained 1·8 milligrams of cobalt. 7·15 grams of fæces excreted during the last 24 hours were found to contain about 100 milligrams of cobalt. Obviously a large amount of the cobalt passes directly through the alimentary canal, while of the absorbed portion considerable is eliminated through the urine, much more indeed than the small amounts found in the kidneys would appear to warrant.

On analysis of the organs of this rabbit, the following results were obtained:

	Total weight of organ. grams.	Weight of Co. milligrams.	Co per 100 grams of tissue. milligrams.
Liver	66·00	2·6	3·94
Spleen	0·40	0·5	125·00
Kidneys	15·30	0·5	3·27
Heart	10·73	0·1	0·93
Lungs	7·02	1·0	14·24
Muscle of leg	140·00	9·8	7·00
Muscle of back	130·00	4·7	3·61
Brain	8·35	1·5	17·96
Spinal cord	4·10	2·0	48·78

Here, as in many of the preceding cases, the lungs contain a higher percentage of the poison than either the liver or kidneys, while the brain and spinal cord contain a still higher percentage.

With this rabbit, convulsions were noticed shortly before death and the breathing was very labored, as if the respiratory muscles were affected.

An experiment, similar to the preceding, tried with nickel, led to a like result as regards the elimination of the poison. The first 24 hours' urine (25 c. c.) contained 1·6 milligrams of nickel, while the portion (70 c. c.) passed during the 24 hours preceding death contained 8·1 milligrams of the metal. Further, 3·5 grams of fæces excreted during the last 24 hours contained 17·5 milligrams of nickel.

It is obvious from the foregoing that soluble nickel and cobalt salts are possessed of decided toxic properties, but that their poisonous action is somewhat slow and manifested only when comparatively large amounts of the salts are administered. Further, so far as our experiments show, the two salts act very much alike. Both apparently cause death by stopping the action of the heart and also produce more or less disturbance in the alimentary tract, interfering with digestion, producing more or less inflammation of the mucous

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membrane of the stomach and intestines, and causing a more or less persistent diarrhœa. Unlike uranium, these salts have no apparent action on the kidneys or liver, sections of hardened tissue from these organs showing no change of structure. Further, in every case the urine of the poisoned animal was entirely free from sugar and albumin throughout the experiment. Both salts tend to produce a partial paralysis of the extremities, more pronounced possibly with cobalt than with nickel. They enter the circulation quickly, are rapidly distributed to all parts of the body and are in turn more or less rapidly eliminated by the kidneys. Considerable, however, evidently passes directly through the alimentary tract and is excreted through the fæces.

Both salts appear to raise the internal body temperature quite decidedly, the rectal temperature rising even two or three degrees centigrade. The blood vessels of the ears, on the contrary, quickly become constricted under the influence of the salts, and the ears appear white and quite cold.

The storage power of the individual organs is somewhat peculiar. The spinal cord and brain, in the majority of the experiments, stand first in their power of picking up and retaining the nickel and cobalt. This is in close accord with what has already been found with soluble forms of arsenic,* and more recently with strychnine sulphate.† It would have been interesting in this connection to have seen whether, as with arsenic, the form of the poison modifies the relative amount absorbed by the brain and spinal cord, but this we did not have time to try. Again, as with soluble forms of arsenic, the muscle tissue shows in several of the experiments, as Nos. V and VI, a marked affinity for nickel and cobalt, retaining a larger percentage of the metals than either the liver or kidneys. Still more noticeable, in all of the experiments but one, is the much larger amount of poison in the muscles of the back than in the muscles of the leg; a constant difference occurring in a tissue of the same kind, and hardly explainable on the ground of difference in vascularity. A somewhat similar distribution of arsenic in the muscle tissue was found by one of us a few years ago in an arsenical poison case.‡

* Chittenden, Amer. Chem. Journal, vol. v, p. 8, also Studies from Laboratory of Physiological Chemistry, vol. i, p. 141. Scolosuboff, Bulletin de la Société Chimique de Paris, vol. xxiv, p. 125.

† R. W. Lovett, Journal of Physiology, vol. ix, p. 99.

‡ Chittenden, Amer. Chem. Journal, vol. v, p. 12.

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Another striking feature in the storage of nickel and cobalt by the tissues, is the comparatively large amount retained by the lungs and heart, the amount found in these organs generally exceeding the amounts stored up in the liver and kidneys.

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